University of Alberta

Characterization of bison muscle tissue and evaluation of the efficacy of postmortem carcass treatments designed to influence the quality of bison meat

by

Jennifer Anne Marie Janz

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Agricultural, Food and Nutritional Science

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Characterization of bison muscle tissue and evaluation of the efficacy of postmortem carcass treatments designed to influence the quality of bison meat** submitted by **Jennifer Anne Marie Janz** in partial fulfillment of the requirements for the degree of Master of Science.

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Dedication

To women in agriculture
To Loreen Onischuk who unknowingly planted the seeds of inspiration when I was looking for direction
To Jennifer Aalhus for providing life advice and a grand example for successfully combining parenting and research

To my academic friends and colleagues (Mick Price, Bill Guenter, Sam Baidoo, Rick Holley, Alma Kennedy, Doug Veira) who have shimmed my confidence, provided motivation, and looked out for my best interest when I was unsure

To Stephen, my best guy
You have patiently attended countless meetings, classes, research trips, and endured my educational pursuits never complaining and always with interest
I am fortunate to be on your team
I love you

Abstract

Despite a substantial increase in bison production for meat, there is a paucity of information regarding bison carcass and meat quality. The present objective was to characterize carcass, meat quality, nutritional, and biochemical traits of bison muscle tissue and meat and to examine the effects of various postmortem carcass handling techniques on meat quality parameters.

Based on myofibre parameters and intracellular biochemistry, production of bison in a relatively undomesticated state is recommended. Bison carcasses had a greater yield, lower fat, and darker meat colour than comparable beef samples. Tenderness was variable, with a wide range in shear values.

Elevated temperature conditioning of bison carcasses resulted in consistently tender meat with reduced ageing requirement. Blast carcass chilling decreased cooler shrink loss, and when combined with low voltage electrical stimulation, yielded tender samples and would allow rapid product throughput. Results are introductory to the development of a genus specific carcass processing protocol.

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Table of Contents

Chapter 1: General introduction and literature review 1

1.1. Muscle function in the live animal 1

1.2. Gross constituents of muscle 1

1.2.1. Connective tissue fraction 1

1.2.2. Myofibrillar fraction 2

1.3. Component proteins of the myofibrillar fraction 4

1.4. Histochemical myofibre types 5

1.5. Contraction mechanism 5

1.6. Conversion of muscle to meat 6

1.6.1. Biochemical changes 6

1.6.2. Biophysical changes 7

1.7. Importance of muscle to meat conversion to meat quality 8

1.8. Definition of meat and meat quality 8

1.9. Components of meat quality 10

1.10. Meat industry trends 11

1.11. Postmortem carcass handling techniques 12

1.11.1. Ageing 12

1.11.2. Carcass chilling regime 13

1.11.2.1. Influences on cooling rate 13

1.11.2.2. Definition of cold shortening 14

1.11.2.3. Mechanism of cold shortening 15

1.11.2.4. Risk of cold shortening in intact carcasses 16

1.11.3. Elevated temperature conditioning 16

1.11.3.1. Mechanisms of action 17

1.11.3.1.1. Prevention of cold shortening 17

1.11.3.1.2. Activation of autolytic enzymes 17

1.11.3.2. Elevated temperature conditioning influence on meat quality 18

1.11.3.2.1. Tenderness and palatability traits 18

1.11.3.2.2. Appearance scores 18

1.11.3.2.3. Concerns with application of elevated temperature conditioning 18

1.11.4. Blast chilling 19

1.11.4.1. Blast chilling influence on meat quality 19

1.11.4.1.1. Tenderness and palatability traits 19
1.11.4.1.2. Appearance scores 20
1.11.5. Carcass electrical stimulation 20
1.11.5.1. History of the technology 20
1.11.5.2. Electrical parameters and charge distribution 21
1.11.5.3. Mechanisms of action influencing meat quality 22
1.11.5.3.1. Prevention of cold shortening 23
1.11.5.3.2. Activation of autolytic enzymes 23
1.11.5.3.3. Tissue rupture 24
1.11.5.3.4. Non-enzymatic weakening of myofibrillar structure 25
1.11.5.4. Electrical stimulation influence on meat quality 25
1.11.5.4.1. Tenderness and palatability traits 26
1.11.5.4.2. Appearance scores 27
1.12. Practical application of postmortem carcass technologies 27
1.13. Bison carcass handling and the application of alternative techniques 28
1.14. Literature cited 29

Chapter 2: Characteristics of bison as a meat producing animal 35
2.1. Introduction 35
2.2. Materials and methods 36
2.2.1. Experimental animals 36
2.2.2. Slaughter, carcass dressing, and meat sampling 37
2.2.3. Fibre diameter and sarcomere length 38
2.2.4. Shear force measurement 39
2.2.5. Drip loss 39
2.2.6. Composition analysis 40
2.3. Results and discussion 41
2.3.1. Carcass traits 42
2.3.2. Carcass grade 42
2.3.3. Carcass yield 45
2.3.4. Meat quality traits 45
2.3.5. Nutritional quality traits 48
2.4. Conclusion 49
2.5. Literature cited 50

Chapter 3: Histological and biochemical characterization of bison muscle tissue and meat 52
3.1. Introduction 52
3.2. Materials and methods 52
3.2.1. Analysis of glycolytic components 53
3.2.2. Histological analysis 54
3.2.3. Dentition score classification and statistical analysis 55
3.3. Results and discussion 56
3.3.1. Postmortem pH decline and glycogen and lactate concentrations 56
3.3.2. Sarcomere length and fibre diameter 57
3.3.3. Myofibre complement and dimensions 58
3.4. Conclusion 61
3.5. Literature cited 62

Chapter 4: Elevated temperature conditioning of bison carcasses 65
4.1. Introduction 65
4.2. Materials and methods 66
4.2.1. Animals, slaughter, postmortem carcass treatment 66
4.2.2. Carcass and meat quality analyses 66
4.2.3. Statistical analyses 66
4.3. Results 67
4.3.1. Carcass traits 68
4.3.2. Temperature decline 68
4.3.3. Biochemical traits 68
4.3.4. Quality traits 69
4.3.5. Tenderness frequency and ageing 69
4.4. Discussion 71
4.4.1. Temperature decline 71
4.4.2. Weight loss 71
4.4.3. Fibre diameter 72
4.4.4. Biochemical traits 72
4.4.5. Colour 73
4.4.6. Sarcomere length 73
4.4.7. Shear force 75

4.4.8. Tenderness frequency and ageing 75
4.5. Conclusion 76
4.6. Literature cited 77

Chapter 5: Blast chilling and low voltage electrical stimulation treatment of bison carcasses 79
5.1. Introduction 79
5.2. Materials and methods 80
5.2.1. Animals, slaughter, postmortem carcass treatment 80
5.2.2. Carcass and meat quality analyses 80
5.2.3. Organoleptic analyses 81
5.2.4. Statistical analyses 81
5.3. Results 82
5.3.1. Carcass traits 82
5.3.2. Biochemical traits 82
5.3.3. Temperature decline 84
5.3.4. Quality traits 85
5.3.5. Tenderness frequency and ageing 86
5.3.6. Organoleptic analysis 87
5.4. Discussion 88
5.4.1. Biochemical traits 88
5.4.2. Temperature decline 89
5.4.3. Colour 89
5.4.4. Weight loss 90
5.4.5. Fibre diameter 91
5.4.6. Sarcomere length 91
5.4.7. Shear force 91
5.4.8. Tenderness frequency and ageing 92
5.4.9. Organoleptic analysis 93
5.5. Conclusion 94
5.6. Literature cited 95

Chapter 6: General summary and conclusions 98

7. Appendices 100

7.1. Canadian bison carcass grading standards 101

7.2. Tabulated data from analysis of glycolytic components (Chapter 3) 102

7.3. Tabulated data from analysis of glycolytic components (Chapter 4) 102

7.4. Tabulated data from analysis of glycolytic components (Chapter 5) 103

7.5. Postmortem colour development in meat 104

List of Tables

Table 2-1: Summary of Alberta bison statistics 35

Table 2-2: Source information for experimental animals 37

Table 2-3: Bison carcass characteristics and *Longissimus lumborum* quality traits 43

Table 2-4: Comparison of bison and beef cuts (% side weight) and saleable yield 46

Table 2-5: Mean shear value ranges from beef quality studies with various experimental conditions 47

Table 2-6: Analysis of bison *Longissimus lumborum* (N=20) 48

Table 3-1: Histological characteristics (SEM) of bison *Longissimus lumborum* (N=18) myofibres 58

Table 3-2: Combined bison fibre type values (SEM) and comparative beef data 59

Table 4-1: Bison *Longissimus lumborum* quality traits by chill treatment 70

Table 4-2: Absolute shear value (kg) and sarcomere length (μm) ranges by treatment and ageing time 70

Table 5-1: Scoring descriptors for organoleptic analysis characteristics 81

Table 5-2: pH in bison *Longissimus lumborum* at various times postmortem 83

Table 5-3: Glycogen:lactate in bison *Longissimus lumborum* at various times postmortem 84

Table 5-4: Bison *Longissimus lumborum* quality traits by stimulation and chill treatments 86

Table 5-5: Bison *Longissimus lumborum* quality traits by chill treatment 86

Table 7-1: Canadian bison carcass grading criteria 101

Table 7-2: Minimum, mean, and maximum concentrations ($\mu mol \cdot g^{-1}$) of glycogen, lactate, and glucose at various times postmortem in *Longissimus lumborum* of conventionally treated bison carcasses 102

Table 7-3: Mean concentrations ($\mu mol \cdot g^{-1}$) of glycogen, lactate, and glucose at various times postmortem in *Longissimus lumborum* from bison carcasses chilled conventionally (CONV) and with elevated temperature conditioning (ETC) 102

Table 7-4: Mean concentrations ($\mu mol \cdot g^{-1}$) of glycogen, lactate, and glucose at various times postmortem in *Longissimus lumborum* from bison carcasses treated conventionally (CONTROL), low voltage electrical stimulation (LVES), blast chilling (BL), or a combination of low voltage electrical stimulation and blast chilling (LVES/BL) 103

List of Figures

Figure 1-1: Diagrammatic representation of hierarchical arrangement of muscle from whole muscle to myofibre 3

Figure 3-1: Comparative pH in postmortem bison and beef *Longissimus* 56

Figure 3-2: Comparative glycogen and lactate concentrations in postmortem bison and beef *Longissimus* 57

Figure 4-1: Postmortem temperature decline of bison *Semimembranosus* (SM) and *Longissimus lumborum* (LL) 68

Figure 4-2: G:L and pH decline in bison *Longissimus lumborum* 69

Figure 4-3: Tenderness frequency counts of bison *Longissimus lumborum* steaks during ageing 71

Figure 5-1: Postmortem pH decline in bison *Longissimus lumborum* by stimulation and chill treatments 83

Figure 5-2: Postmortem glycogen:lactate (G:L) decline in bison *Longissimus lumborum* by stimulation and chill treatments 84

Figure 5-3: Postmortem temperature decline in bison *Longissimus lumborum* by chill treatment 85

Figure 5-4: Tenderness frequency counts of bison *Longissimus lumborum* steaks during ageing 87

Figure 5-5: Organoleptic analysis of bison *Longissimus lumborum* 87

Figure 7-1: CIE L*a*b* colour space 106

CHAPTER 1

General introduction and literature review

1.1. Muscle function in the live animal

Muscle is a specialized tissue with a unique architecture that allows coordinated contraction to generate force and permit movement. In the living animal, the system is able to convert chemical energy into mechanical work (McCormick 1994). A thorough examination of postmortem meat quality must logically begin with a description of muscle. Since the tissue once served a biological function in the animal from which it was derived, a basic review of structure and function is appropriate.

1.2. Gross constituents of muscle

A generalization of muscle tissue structure will reveal that it has two main components: connective tissue, primarily collagen, and myofibrillar or contractile proteins.

1.2.1 Connective tissue fraction

Muscle tissue is encompassed by a continuum of connective tissue that contains extracellular elements, maintains structural integrity, forms attachments among muscles and between muscle and bone, and permits the transmission of force generated by the muscle (McCormick 1994). Connective tissue content varies amongst muscles with the postural muscles such as the *Psoas major* and *Longissimus thoracis et lumborum* (previously referred to as *Longissimus dorsi*) containing relatively little and muscles involved in locomotion, for example the *Biceps femoris* and *Semitendinosus*, containing relatively more (Miller 1994).

Collagen is the primary protein in the connective tissue within and surrounding muscle and constitutes 2-6% of muscle on a dry matter basis (McCormick 1994). Collagen biosynthesis involves the formation of a high tensile strength fibre based on collagen fibrils bound by trivalent crosslinkages (McCormick 1994). Strands of the primary tropocollagen sequence, containing one third glycine and one third proline and hydroxyproline, form triple helices that are further organized into an array of repeating fibrils with a quarter stagger arrangement. Hydroxyproline is an amino acid unique to collagen and is the basis for connective tissue assay. The covalent crosslinks between and within fibrils, increase in number and stability with the physiological maturity of the animal (Shimokomaki et al. 1972) contributing to increased toughness with increasing animal age. According to Marsh (1977), youthful crosslinkages are unstable when exposed to denaturing conditions, while crosslinks in mature animals are more heat resistant.

Shorthose and Harris (1990) demonstrated that tenderness of the *Psoas major*, a muscle with low connective tissue strength (Herring et al. 1965), was almost unaffected by age while the tenderness of the *Biceps femoris*, a muscle with high connective tissue strength, tripled in toughness over the same age range. The indication is that collagen quality, not quantity, is important in terms of meat quality.

While the connective tissue in meat certainly exerts some influence on eating quality (Smith et al. 1976), the postmortem changes to the degree of those associated with the myofibrillar fraction of meat have not been observed in the connective tissue fraction (Marriott and Claus 1994). In addition, the usefulness of connective tissue content, as indicated by hydroxyproline content, as a predictor of tenderness is limited to cuts of meat containing a relatively high proportion of connective tissue (Parrish et al. 1962). As such, the main focus of tenderness improvement strategies have focussed on the myofibrillar portion (Marriott and Claus 1994) and the contribution of connective tissue has often been relegated to the role of "background toughness" (Newbold and Harris 1972; Olsson et al. 1994). In the present review, discussion of connective tissue will be limited.

1.2.2. Myofibrillar fraction

The myofibrillar or contractile component of muscle in the live animal allows generation of force by synchronized contraction. Postmortem, the myofibrillar fraction is the major textural component (Davey and Gilbert 1967) and provides the unique flavour of meat. As compared to connective tissue, the myofibrillar fraction is more amenable to postmortem modification (Marsh 1977) by manipulating environmental conditions.

Muscle tissue is an array of proteins with a precise geometry, aligned to form a unique and highly structured arrangement of compounded components (Figure 1-1). Each structural level is enclosed in its own connective tissue wrapping. Molecular myofilaments form contractile units that together make up myofibrils. Myofibrils are closely associated and enclosed within a cell membrane to form myofibres or muscle cells. Each muscle cell is surrounded by endomysium, a connective tissue layer. Fibres are packed into bundles or fasciculi enclosed by perimysium. Entire muscles, then, are composed of successive fasciculi enclosed in an epimysial layer terminating at a myotendinous junction.

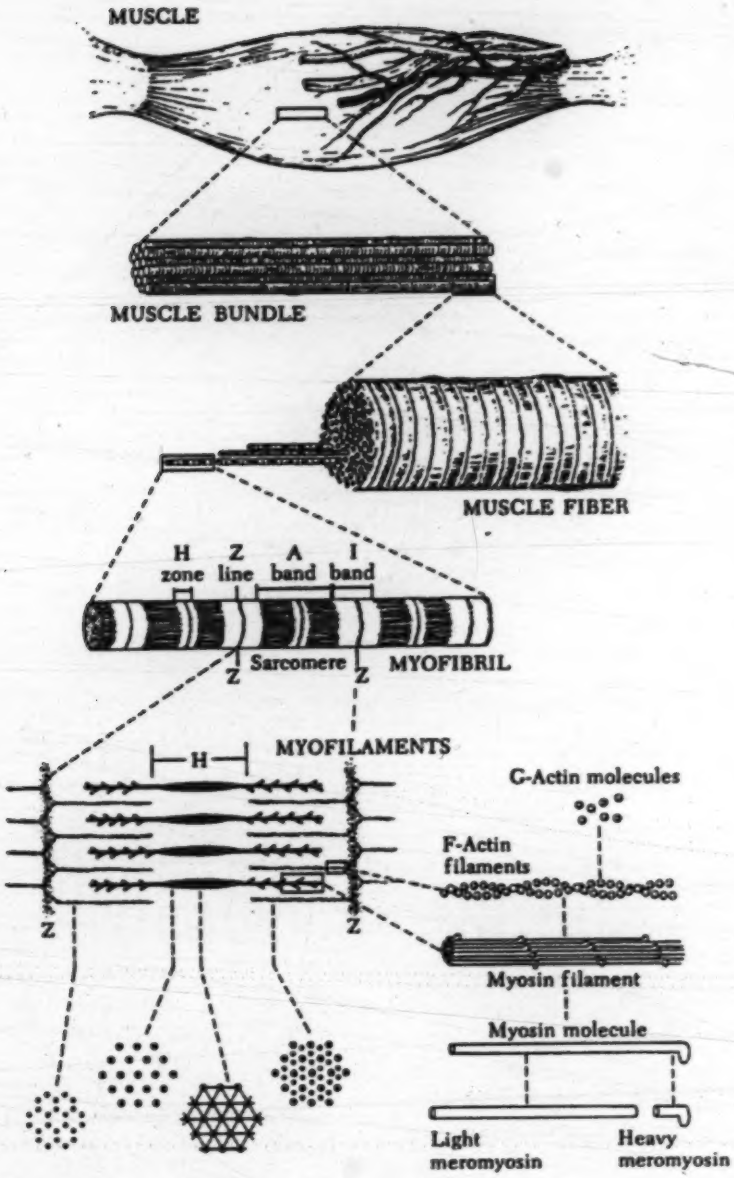

Figure 1-1. Diagrammatic representation of hierarchical arrangement of muscle from whole muscle to myofibre (Forrest et al. 1975).

1.3. Component proteins of the myofibrillar fraction

The basic and repeating unit of muscle contraction, the sarcomere, is composed of a precise geometric arrangement of proteins that form a contractile mechanism. One sarcomere is defined as the distance between two adjacent Z disks and its length changes with contraction state. Two sets of thin filaments interdigitate a set of thick filaments with six thin filaments surrounding each thick filament (Figure 1-1). The thin filaments form the length of the I (isotropic) band with this band traversing the Z disk. The length of the thick filaments defines the A (anisotropic) band. The H zone region of the A band is the area with no projections along the thick filaments. The M line bisects the H zone and represents the region of connection of back to back tail ends of the thick filaments. The portion of the H zone not overlapped by the I band is referred to as the pseudo-H zone. During contraction, the A band length remains unchanged while the pseudo-H zone and I bands decrease in length with increasing overlap between thin and thick filaments.

The thin filament is a complex of three proteins: actin, tropomyosin, and troponin. Actin is a double helical arrangement of F-actin (F: filamentous), a string of repeating G-actin (G: globular) monomers. Each monomer has six binding sites: 2 for chain formation, 2 for helix formation, and one each for binding myosin and tropomyosin. Tropomyosin is composed of two polypeptide chains in helical arrangement. Troponin has three subunit proteins: T, C, and I. Troponin-T joins troponin to tropomyosin, troponin-C is the calcium sensitive subunit, and troponin-I is inhibitory and binds to both subunit C and to actin.

The thick filament is composed of 300-400 myosin molecules of varying isoforms. Each molecule has a long tail region made of helically arranged polypeptide chains with two globular heads located at one end. Associated with the head region are light protein chains. Myosin has both structural function and enzyme activity. The head regions have ATPase activity that varies with the type of associated light chain.

The Z disk is a protein lattice through which the F-actin filaments enter and exit to traverse two sarcomeres along the length of one I band. A number of small structural proteins, including titin, nebulin, and desmin, play an integral role in maintaining the precise arrangement of the above mentioned components of the sarcomere. Titin is a highly elastic filament (McCormick 1994) that joins myosin to the Z disks in order to maintain the central location of the thick filaments in the sarcomere (Takahashi 1996) and to maintain the thin and thick filaments in register (Pearson and Young 1989). Nebulin is also closely associated with the thin filaments, forming a link to the Z disk (Takahashi 1996). Desmin is located within and between adjacent Z disks and serves to maintain lateral association (McCormick 1994).

1.4. Histochemical myofibre types

Skeletal muscle is not composed of homogeneous myofibres. Differentiation of muscle cells occurs during embryonic formation and, once fibres have reached a functional state, differentiation continues in order to meet the demands placed on the tissue (Swatland 1984, McCormick 1994). A multitude of fibre classification systems exists, based on fibre appearance, physiological behaviour, biochemical properties, and histochemical staining properties (Pearson and Young 1989). Although a continuum of traits exists, individual fibres are often referred to as exhibiting slow or fast contraction, having an oxidative or glycolytic metabolism, and being red or white in appearance (McCormick 1994). Fibre type differences are due to the variation in protein isoforms possible for virtually all constituents of the myofibrillar complex, as well as the metabolic enzymes of muscle tissue (McCormick 1994). Given the extensive range of potential isoform and enzyme combinations, it is obvious that any classification system is simply a crude approach at approximating muscle composition.

1.5. Contraction mechanism

The sliding filament theory is the generally accepted model for muscle contraction and force generation. In general, within each sarcomere the two sets of thin filaments move along the thick filament towards each other in a rachet-like fashion resulting in an increased interdigitation of actin and myosin. The movement is stimulated by nervous impulse, permitted by calcium ion concentration, and powered by ATP hydrolysis.

McCormick (1994) described the transmission of a nervous impulse from the motor end plate across the sarcolemma and into the interior of the cell by the transverse tubule (T-tubule) system. The T-tubule system meets the sarcoplasmic reticulum (SR) over the A-I band junction where actin-myosin interaction begins. The end result of the nervous impulse is the release of calcium ions from the SR into the sarcoplasm, increasing the concentration from 10^{-7} (off state) to 10^{-5} (on state). Once the impulse passes, the SR reaccumulates the ions (Briskey and Fukazawa 1971).

The release of calcium ions to reach the "on state" concentration exerts an influence on the calcium sensitive troponin-C subunit. The resulting conformational changes cause an alteration of the linkages between troponin-T and tropomyosin, and troponin-I and actin. The myosin binding sites on actin monomers, normally shielded by tropomyosin in the "off state", are exposed allowing myosin-actin interaction.

McCormick (1994) summarized the events in the sliding filament model:

- ATP associated with the myosin head is hydrolysed and the contractile cycle is initiated
- If binding sites on actin are available ("on position" relative to calcium ion concentration), myosin and actin form a crossbridge
- Myosin head region swivels from 90° to 45°, relative to the axis of the thick filament causing movement of the thin filament along myosin for the length of one crossbridge
- ADP and P_i are released and a new ATP binds to the myosin head
- Myosin dissociates from actin and swivels back to the 90° "resting" position
- As the new ATP is hydrolysed, the cycle is repeated

The release of actin from myosin is an ATP dependent event. The presence of the energy molecule is required at the binding site on myosin, however, its hydrolysis is not necessary. This ATP requirement has important implications in the development of rigor mortis and ultimate sarcomere length and will be discussed further in relation to the conversion of muscle to meat.

1.6. Conversion of muscle to meat

At slaughter, meat animals are first rendered unconscious then immediately shackled and exsanguinated. The ensuing loss of blood circulation initiates a cascade of intracellular events ultimately resulting in the establishment of rigor mortis and the conversion of muscle tissue to meat. The changes that occur, while interrelated, can be placed into two categories: biochemical changes involving cellular energy compounds, and biophysical changes in the contractile proteins.

1.6.1. Biochemical changes

Loss of oxygenated blood flow while cells remain metabolically active necessitates the alteration of metabolism from aerobic to anaerobic under anoxic conditions. Lack of circulation also halts the removal of metabolic by-products from the cell (Lawrie 1998). Faustman (1994) summarized aerobic and anaerobic energy production in the following equations:

Aerobic ATP production via aerobic glycolysis, Kreb's cycle and electron transport chain:

$$1 \text{ glucose} + 36 \text{ Pi} + 36 \text{ ADP} + 6 \text{ O}_2 \rightarrow \mathbf{36 \text{ ATP}} + 6 \text{ CO}_2 + 42 \text{ H}_2\text{O}$$

Anaerobic ATP production via anaerobic glycolysis:

$$1 \text{ glucose} + 3 \text{ Pi} + 3 \text{ ADP} \rightarrow \mathbf{3 \text{ ATP}} + 2 \text{ lactate} + \mathbf{2 \text{ H}^+} + 3 \text{ H}_2\text{O}$$

Far less ATP is produced by the less efficient anaerobic system, while sarcoplasmic ATPases continue to function postmortem (Lawrie 1998). ATP is depleted as the rate of consumption overtakes the rate of production. Binding of ATP at the myosin head region is required for release of actin from myosin. The loss of ATP over time postmortem results in concomitant increase in unreleased actomyosin crossbridges (Faustman 1994).

Anaerobic glycolysis produces lactic acid that accumulates intracellularly with the lack of a waste removal system and dissociates to lactate and hydrogen ions. The result is a pH decline from near normal resting pH ~7.3 (Newbold and Harris 1972) to ultimate pH (pH_u) in the range of 5.4-5.7 (Murray 1995). Glycolysis is terminated when the enzymes involved are inactivated by the acidic environment (Pearson and Young 1989).

Glycolysis and the resulting rate of pH decline are temperature dependent (Newbold and Harris 1972; O'Halloran et al. 1997) while the extent of pH decline, described by pHu, depends on initial concentration of energy compounds including glycogen and ATP. Rate of glycolysis is faster at higher muscle temperature as affected by ambient temperature and the insulative value of lean thickness and fat cover (Faustman 1994). Both rate and extent of pH decline are related to tenderness (Newbold and Harris 1972), exerting an influence on endogenous proteolytic enzyme activity (Faustman 1994; O'Halloran et al. 1997) and the contraction state of myofibrillar proteins.

1.6.2. Biophysical changes

The postmortem decline in pH accompanying biochemical changes causes a decrease in membrane integrity of the sarcoplasmic reticulum and mitochondria resulting in release of calcium ions (Aalhus 1995). Availability of free calcium and ATP facilitate actin/myosin interaction and muscular contraction. As ATP concentration decreases over time postmortem, there is a locking of the actomyosin complex. The number of rigor links increases until rigor mortis is fully developed. According to Bodwell et al. (1965) rigor onset occurs at 12-15 hours postmortem and is complete by 24 hours when ATP concentration is exhausted; however, the process is temperature dependent. Under typical cooler conditions near 2°C, approximately 80% of ATP is depleted by 12 hours postmortem (Busch et al. 1967). Development of isometric tension in the muscle tissue, greatest at 2°C, is characteristic of the postmortem period leading up to full rigor development (Busch et al. 1967). Fully developed rigor mortis is defined by the formation of actomyosin such that thin and thick filaments are locked together and extensibility reaches its lowest point (Swatland 1984; Faustman 1994; Aalhus 1995; Lawrie 1998). This

physical state is accompanied by an ATP concentration at or near zero (Newbold and Harris 1972; Swatland 1984). At this point, muscle is fixed in whatever state of contraction occurred at the time of ATP depletion (Newbold and Harris 1972). Contraction state is a determinant of ultimate eating quality and is influenced by restraint imposed by skeletal attachments and by the time/temperature course to rigor mortis.

1.7. Importance of muscle to meat conversion to meat quality

Pre-rigor conditions to which carcasses are subjected influence the above described chemical and physical changes. Carcass cooling temperature, especially, influences chemical reaction rates which in turn affect pH decline and development of contraction state. Rapid cooling can induce supercontraction and the phenomenon of cold-shortening or cold-toughening (refer to Section 1.11.2.2.). Excessively slow chilling made possible by high ambient temperature and/or well insulated carcasses can result in rigor- or heat-shortening (refer to Section 1.11.3.2.1.), a forceful contraction during rigor mortis development. Unrestrained tissue is free to shorten to the full extent permitted by remaining energy stores and potential actin/myosin linkages.

According to Olsson et al. (1994) myofibrillar toughness depends on the course to rigor mortis as influenced by postmortem time-temperature gradient. It is clear that postmortem, especially pre-rigor, handling of carcasses is a major determinant of meat quality.

1.8. Definition of meat and meat quality

Having described the components of muscle and their conversion to meat, the next logical step in the discussion of meat quality is to define what is meant by quality. Jeremiah (1978) defined meat as "skeletal muscle from animals, including the connective tissue and fat naturally associated with this muscle". More elaborate and inclusive definitions exist, but for the purpose of a review on meat quality and postmortem technologies this definition is sufficient as it includes the major components of meat previously discussed.

The definition of quality is not as clearly defined. A lack of consensus arises because the definition of the term "quality" depends on the purpose of the definition and who defines it. There are different steps in the definition of quality during the production of meat (Hofmann 1994): animal quality, carcass quality, meat quality, and meat product quality. At any given point in the production chain definition of quality must serve to describe the characteristics of the product in terms of the target interest group. As the commodity changes ownership, the criteria for defining quality must change as the product is transformed. The definition must facilitate to

trade within the meat industry and must satisfy the description of consumer preferences (Asghar and Pearson 1980). A producer, a meat scientist, a meat processor, and a consumer will make very different use of the definition of quality (Hofmann 1994).

Carcass appraisal, and the subsequent value assigned by grade, are important for recognition of growth efficiency (Yeates 1965) and are based on an estimate of lean yield. In this situation, quality comes to mean quantity (Hofmann 1994). This use of the term is appropriate for the assignment of value to the carcass with the intent of facilitating trade; however, carcass quality should not be confused with meat quality. Proportion of lean, fat and bone determine carcass quality, but this is not indicative of eating quality (Asghar and Pearson 1980). Crouse et al. (1978) determined that grade accounted for only 2-3% of the variation in taste panel tenderness score and 6-8% of variation in taste panel acceptability.

The category of meat quality can be subdivided into visual quality factors (colour and texture) and eating quality factors (tenderness, juiciness, flavour) (Asghar and Pearson 1980). The evaluation of these meat quality characteristics can be made objectively and/or subjectively. Objective evaluation makes use of instrumental measurement and yields repeatable results (Asghar and Pearson 1980). The material properties of the product are described neutrally and lack positive or negative connotation. Subjective quality evaluation makes use of the human senses of taste, smell, and touch (Asghar and Pearson 1980) and are connected with positive perception based on consumer appreciation (Hofmann 1994). Objective quality evaluation produces a description of meat quality while subjective quality evaluation describes quality meat (Hofmann 1994). For scientific use, quality must be defined objectively, based on the characteristics of the product and independent of public opinion (Hofmann 1994). While there is a trend towards objective description, complete disregard for subjective sensory evaluation may lead to error greater than that encountered when using subjective assessment alone (Asghar and Pearson 1980). The action of mastication and the sensory aspects of mouthfeel are complex and, thus far, cannot be reproduced by any mechanical device designed for objective meat quality evaluation. Objective observations are sometimes only vaguely connected with the organoleptic quality they are designed to measure (Asghar and Pearson 1980). It is essential, then, to consider both subjective and objective meat quality evaluation methods. To make subjective sensory taste panel analyses credible it is essential to indicate variability amongst panel members (Blumer 1963) in an attempt to use the human senses as objective tools.

1.9. Components of meat quality

Jeremiah (1978) listed the factors determining saleability and consumer acceptance, hence meat quality, as colour, moisture retention, tenderness, and flavour. Asghar and Pearson (1980) extended this concept by subdividing meat quality into appearance and eating quality or palatability factors. While visual appearance, especially the colour of lean and fat, is crucial in influencing a consumer decision to purchase, eating quality is essential for maintaining customer satisfaction. A satisfied customer will repurchase a product perceived to be of high quality. Palatability is defined as agreeableness of a taste and is a complex of sensations resulting from stimulation of the senses of odour, taste, and feel in addition to ease of mastication (Blumer 1963). There is general agreement in the literature that eating satisfaction is based on the interaction amongst tenderness, juiciness, and flavour (Koohmaraie 1996).

Juiciness and flavour are components in the eating quality equation, but are not nearly as influential as tenderness. Juiciness is the liquid detectable during the chewing of meat and is associated with intramuscular fat content. Fat flavour stimulates the flow of saliva during mastication, resulting in the sensation of juiciness. According to Blumer (1963), approximately 16% of variance in juiciness is attributable to intramuscular fat content. Flavour is difficult to define (Blumer 1963) and is the result of the combination of compounds in the tissue and those produced by the Maillard reaction during cooking.

Tenderness is the most important quality attribute (Olsson et al. 1994) exerting the greatest impact on acceptability (Jeremiah and Martin 1978) as rated by consumers (Lawrie 1998). The definition of tenderness involves the ease of penetration of meat by the teeth, the ease with which meat fragments, and the amount of residue remaining after chewing (Lawrie 1998). Unfortunately, there exists a serious lack of uniformity in this trait (Marriott and Claus 1994). For Canadian consumers inconsistent meat quality, especially tenderness, is a concern (Aalhus et al. 1992).

Tenderness variability can be classified into two separate categories: inherent and induced variability. Inherent variation in tenderness is a result of age and anatomical location influences on existing tissue characteristics. Collagen undergoes continuous crosslinking, with the number of linkages increasing with animal age (Asghar and Pearson 1980) yielding a relationship between age and connective tissue related toughness (Miller 1994). Increasing muscle fibre size may also be related to age with size increasing with age and affecting perceived meat texture. Anatomical location will affect connective tissue content. Muscles involved in locomotion contain more connective tissue than postural support muscles (Miller 1994). Swanson et al. (1965) demonstrated variability in muscle fibre size along the *Longissimus*. The

smallest fibres were located over the 12th rib with size increasing both posterior and anterior to this location. These examples demonstrate the potential for variability within a carcass and even within one cut of meat.

Induced tenderness inconsistencies are the result of the influence of processing techniques over which control can be exerted. Postmortem myofibrillar changes, especially in the pre-rigor period, influence the tenderness of the resulting meat (Newbold and Harris 1972) by exerting an influence on the biochemical and biophysical changes occurring at the cellular level. Time and temperature conditions affect rate of carcass cooling which in turn influences the rate of anaerobic glycolysis and activity of endogenous proteolytic enzymes (O'Halloran et al. 1997). Cooling rate also affects the time course to rigor mortis (Olsson et al. 1994) and, by influencing biochemical processes, the extent of actin/myosin interaction (Asghar and Pearson 1980) ultimately determining contraction state of muscle and influencing tenderness of meat. Normal cooling practices can yield more than a three-fold range in toughness (Smulders et al. 1990). Inappropriate processing conditions can induce toughening in potentially tender meat (Newbold and Harris 1972).

1.10. Meat industry trends

According to Jones (1989), the Canadian meat industry has shown a trend towards the processing of leaner carcasses in large, single species plants focused on increased product throughput. The result has been a saving of energy input and labour requirement to chill and trim lean carcasses. At the same time, however, concern has arisen over a decline in meat quality from lean carcasses. According to Zamora et al. (1996), the consumption of beef has declined since the early 1980s. This has been due in part to the variability in organoleptic quality, especially tenderness (Zamora et al. 1996).

The meat quality properties of most interest to the consumer are those most strongly affected by conditions imposed in the perimortem period (O'Halloran et al. 1997). Very little variation in tenderness of the *Longissimus* (standard muscle for meat quality evaluation) exists at slaughter, but differences in reaction to postmortem conditions induce inconsistency in meat quality (Koohmaraie et al. 1996). It is imperative that processing trends parallel the trend in carcass type in order to ensure appropriate carcass handling techniques are used. For example, cooler conditions are geared towards the largest, fattest carcass likely to be encountered and these conditions would likely induce cold toughening in a smaller carcass or one with less finish. Faustman (1994) indicates that selection of lean livestock results in a predisposition towards cold shortening. Manipulation of post mortem conditions can produce the physicochemical

characteristics (Asghar and Pearson 1980) required to increase the level and consistency of tenderness (Fisher et al. 1994) and allow the production of tender meat from lean carcasses. A fine balance must be struck if lean product quality is to be maintained while adopting alternative techniques without compromising increased throughput.

Solving the problems of variation in tenderness and inconsistency in product quality has been identified as a top priority in the meat industry (Koohmaraie et al. 1996). This is a legitimate concern given the recent, and seemingly divergent, industry and consumer trends.

1.11. Postmortem carcass handling technologies

The goal in the application of any postmortem technology is to improve meat quality. Most often this refers to an increase in tenderness, but improvement in other quality parameters may also occur. The conversion of muscle to meat is a dynamic event. The series of metabolic and structural changes that occurs postmortem, coupled with the responsiveness of the myofibrillar component of muscle to modification, creates a window of opportunity for the manipulation of meat quality.

1.11.1 Ageing

The holding of carcasses at refrigeration temperature for an extended period of time, known as ageing, is a traditional treatment known to result in tenderization. A more modern practice is to age product (primal or sub-primal cuts) in vacuum package bags for the duration of the marketing and transportation process, a period that may be as short as three days (Aalhus 1995). Koohmaraie (1996) suggested that the maximum benefit of ageing of beef is obtained after 10-14 days.

Zamora et al. (1998) stated that ageing is one of the most important factors governing meat tenderness and that this process likely involves proteolysis. Changes in tenderness during ageing are associated with the myofibrillar portion of muscle (Purchas 1972) with little proteolytic effect on connective tissue (Lawrie 1998). The gradual tenderizing that occurs during ageing, however, is not due to the dissociation of the actomyosin complex (Lawrie 1998), but, rather, is the result of increased myofibrillar protein solubility (Aalhus 1995) in addition to a host of other structural changes. Other physical changes that have been observed during postmortem ageing include a loss of tensile strength (Davey and Gilbert 1967), and a weakening of fibre structure with a progressive decrease in fibre fragment size (Jeremiah and Martin 1978) until few intact sarcomeres remain (Aalhus 1995).

Postmortem ageing using a relatively higher ambient temperature will result in a similar degree of tenderization in a shorter period of time as compared to ageing at a lower temperature (Lawrie 1998). While the risk of microbial spoilage increases in direct proportion to the time/temperature conditions (Lawrie 1998), this risk can be controlled with the adherence to strict hygiene standards and by ageing product in vacuum sealed bags. Extended storage of bulk product, however, can result in the inefficient use of cooler space. With the ultimate goal of improving meat quality, alternative carcass handling techniques to traditional cooler ageing have been developed.

1.11.2. Carcass chilling regime

The rate of carcass cooling in the early postmortem period is a major determinant of meat quality due to its effects on both biochemical and biophysical processes. Tenderness, especially, is determined by internal muscle temperature within first 24 hours postmortem (Dutson et al. 1975). The use of inappropriate processing techniques, not uncommon in commercial practice, can cause toughness in potentially tender meat (Newbold and Harris 1972). Carcass cooling rate has a potent influence on glycolytic rate, the resulting rate of pH decline, and proteolytic enzyme activity (Smulders et al. 1990; O'Halloran et al. 1997). Rate and extent of pH decline are related to tenderness (Newbold and Harris 1972) and cannot be considered independently of cooling rate (Martin et al. 1983). Cooling rate also influences the physical configuration of pre-rigor muscle, contraction state being an important factor in tenderness (Herring et al. 1965). The ultimate overlap of actin and myosin filaments affects tenderness (Marsh and Carse 1974). Too early exposure to cold can produce disastrous effects on tenderness (Locker et al. 1975) due to the effects of cold shortening, a major determinant of tenderness in early pre-rigor period (Smulders et al. 1990).

1.11.2.1. Influences on cooling rate

Early research linked the improved tenderness of fat versus thin carcasses to the increased palatability derived from greater marbling (Smith et al. 1976). Indeed, Ramsbottom and Strandine (1949) observed that samples from Choice grade carcasses were more tender than those from the Commercial grade and concluded that a higher fat content directly influenced palatability. More recent investigation has also implicated subcutaneous fat cover and marbling as contributors to tenderness. The influence is now recognized to be indirect and owing to the insulative value of intramuscular fat and overlying tissue moderating the effects of carcass cooling rate.

Ambient conditions (temperature, humidity, air velocity) are obvious contributors to carcass cooling rate (Marsh et al. 1968); however, inherent carcass characteristics (size, depth of lean and fat) are just as influential (Lochner et al. 1980) and affect heat exchange and postmortem carcass temperature decline (Aalhus 1995). The confounded effects of carcass weight and fatness decrease "animal heat" dissipation and in the process improve tenderness by lessening sarcomere shortening and enhancing release, activity, and duration of activity of proteolytic enzymes (Smith et al. 1976).

Toughness is determined in the first few postmortem hours and is closely related to chill rate (Marsh 1977), especially where fat cover is inadequate to prevent sarcomere shortening (Marsh and Leet 1966) due to cold exposure. Normal cooling practices can yield a three fold range in toughness (Smulders et al. 1990), especially in the loin and outer layer of the round (Woltersdorf 1988). Marbling has an insulatory effect and reduces the severity of cold shortening under rapid or low temperature chilling conditions (Smith et al. 1976).

1.11.2.2. Definition of cold shortening

Locker (1960) noted the toughness of shortened muscle in the first paper reporting the shortening/toughening relationship. Following this preliminary work, Locker and Hagyard (1963) first defined cold shortening as a shortening of muscle due to cold temperature, suggesting that cold stimulation instigated the shortening response. Shortening was observed to be minimal (<10%) in the 14-19°C range and was maximized at 0°C (~48%), near conventional cooler temperature. Further investigation of the phenomenon led to a refined definition and the description of conditions surrounding cold shortening. Smulders et al. (1990) defined cold shortening as a response in pre-rigor muscle as internal temperature declines towards 0°C, shortening being greater with a faster temperature decline and slower glycolysis. The cold shortening contraction is described as large and slow, sluggish in comparison to physiological response to nervous stimulation (Buege and Marsh 1975). Cold shortening is most likely to occur in the temperature range of -2 to 10°C (Marsh et al. 1968), while intracellular ATP concentration and pH are still high (Nuss and Wolfe 1980-81). Chrystall and Devine (1985) indicated that cold shortening will not occur at pH <6.0. Cold shortening commences within the first 3 hours postmortem and continues until development of rigor mortis is complete at about 16-24 hours postmortem (Davey and Gilbert 1974). Once provoked to shorten, muscle will contract to the fullest extent permitted by skeletal restraint (Smith et al. 1976).

Cold shortening can cause a 4-5 fold increase in the force required to bite through a meat sample (Marsh 1977). There is a general consensus in the literature that peak toughness,

represented by resistance to shearing force, occurs when shortening reaches 40% of initial length (Marsh and Leet 1966; Davey et al. 1967; Marsh and Carse 1974; Marsh et al. 1974). This peak in toughness is independent of the shortening mechanism and is determined by the physical state of the myofilaments (Marsh and Leet 1966).

While the effects of cold shortening are reversible, the ability to recover decreases as time postmortem increases. Locker and Hagyard (1963) observed a nearly complete recovery of excised muscle length at 3 hours postmortem, but by 9 hours postmortem when cold shortening had reached about 40%, only a 32% recovery was observed. When replaced in a cold environment after length recovery, cold shortening recommenced. This sensitivity to temperature was lost once rigor mortis was fully established; cold shortening was no longer reversible.

1.11.2.3. Mechanism of cold shortening

Cold shortening is initiated by a postmortem influx of calcium ions to the sarcoplasm. The contraction mechanism is activated where sufficient ATP is present to fuel the reaction. As muscle temperature declines between 15° and 0°C there is a 30-40 fold increase in calcium concentration (Davey and Gilbert 1974). There is convincing evidence, however, that temperature decline merely coincides with calcium influx and that the actual stimulus for ion release is anoxia causing a response in mitochondria (MT) (Buege and Marsh 1975; Marsh 1977; Cornforth et al. 1980). The free calcium made available in the sarcoplasm is immediately recaptured by the sarcoplasmic reticulum (SR); the membrane integrity of this organelle is important in the moderation of intracellular calcium concentration. The ability of SR to release and recapture calcium is both temperature and pH dependent (Cornforth et al. 1980). At low temperature SR is less able to recapture free calcium (Buege and Marsh 1975) and once pH <5.5-5.0 is reached, this ability is inactivated entirely (Cornforth et al. 1980). Under normal physiological conditions, MT are able to reaccumulate calcium, but the anoxic environment overrides this function (Cornforth et al. 1980). Once calcium is released by MT, cold shortening will occur at temperatures low enough to prevent compensatory calcium uptake by temperature sensitive SR (Buege and Marsh 1975).

The reversible nature of cold shortening depends on the temperature sensitive nature of SR and its ability to accumulate and release calcium under relatively warm and cool conditions, respectively (Cornforth et al.1980). The role of MT in the reversal of cold shortening is negligible since conditions remain anoxic in postmortem tissue regardless of muscle temperature. According to Cornforth et al. (1980), pre-rigor pH and ATP concentration are permissive

conditions. Once rigor is achieved, low pH inactivates SR function and reversal of shortening is not possible.

1.11.2.4. Risk of cold shortening in intact carcasses

The original cold shortening experiments were carried out using excised muscle tissue, but extrapolation of these results to intact carcasses is reasonable (West 1979) despite criticism (Jeremiah 1978) of early work. Using excised bovine *Sternomandibularis*, Marsh and Leet (1966) demonstrated that shortening could occur without an overall change in muscle length. Portions of muscle samples, firmly clamped to fix overall length, were wrapped with several layers of paper to provide insulation. After cooling for 48 hours at 2°C, appreciable shortening had occurred in the unwrapped zones while regions having been insulated were stretched by a corresponding amount. Once cooked and assessed objectively for tenderness, shortened samples were found to be significantly tougher than their stretched counterparts. The conclusions from this study were broadened to include muscles of an intact carcass. The hypothesis stated that muscle firmly fixed to the skeleton could display regions of shortening compensated for by other areas of lengthening where fat cover, bone, or greater muscle depth provides insulation against cold exposure.

This theory was validated by later work. Muscle firmly attached to the carcass was capable of localized shortening in response to differential chilling rate along its length (Locker et al. 1975; Chrystall and Devine 1985). According to Bouton et al. (1973) conventional chilling at 0-1°C could reduce tenderness in certain muscles of the intact carcass. The *Longissimus* is especially susceptible because of its thin, narrow conformation, superficial location away from other muscle groups, and the resulting rapid cooling rate (Powell et al. 1996). Without adequate external fat cover, the *Longissimus* is prone to sustaining shortened sarcomeres and decreased proteolytic enzyme activity (Smith et al.1976). Work with intact carcasses indicated the need for the development of a technology to avoid the detrimental toughening associated with cold shortening.

1.11.3. Elevated temperature conditioning

Maintaining muscle temperature outside of the cold shortening temperature risk zone can avoid the detrimental effects of cold shortening on tenderness. According to the general "10 in 10" rule, internal muscle temperature should remain above 10°C within the first 10 hours postmortem, and this can be achieved with the use of elevated temperature conditioning (**ETC**). ETC is defined as the holding of dressed carcasses above normal chilling temperature for a period

of time prior to subsequent cooling (West 1979; Marsh et al. 1980-81). Elevated temperatures typically range from 15° to 20°C, although may be as high as 37°C, and are generally applied for 3 to 24 hours. The time/temperature treatment is applied to pre-rigor muscle still capable of shortening in response to cold temperature stimulus (Bouton et al. 1973) and serves to prevent toughening and promoting tenderization without the use of costly refrigeration (Smith et al. 1971; Bouton et al. 1973; Parrish et al. 1973).

1.11.3.1. Mechanisms of action

1.11.3.1.1. Prevention of cold shortening

Elevated temperature conditioning is a means of hastening rigor onset (Chrystall and Devine 1985) such that development of rigor is near completion at the time of cold exposure. According to Marsh and Leet (1966), the extent of shortening resulting from chilling temperatures decreases as time between slaughter and cold exposure increases. If cold shortening prevention is to be exploited, ETC must be applied to carcasses in the pre-rigor state.

1.11.3.1.2. Activation of autolytic enzymes

Early studies using ETC attributed tenderizing effects solely to prevention of cold shortening. As examination of the technique progressed, however, activity of proteolytic enzymes was implicated (Parrish et al. 1973; Dutson et al. 1975; Moeller et al. 1976; Dutson et al. 1977; Yates et al. 1983; Dutson and Pearson 1985). Elevated temperature during the pre-rigor period prompts acceleration of postmortem metabolism because glycolytic enzymes are permitted to function near ideal conditions. The result is the development of a low pH environment while high temperature prevails, a situation in which activity of lysosomal proteases (cathepsins) is optimized. Catheptic activity is favoured over that of neutral proteases (calpains) because of the early, rapid pH decline (Yates et al. 1983). Moeller et al. (1976) reported rupturing of lysosomes in ETC samples resulting in release, and enhanced and prolonged activity of Cathepsin C and β-glucuronidase. These conditions were reached in conventionally chilled samples at a later time postmortem indicating that differences in tenderness between treatments results from elevated enzyme activity in the early postmortem period. Yates et al. (1983) suggested that in addition to promoting enzyme activity, the low pH conditions result in a conformational change in myofibrillar proteins making them more susceptible to proteolytic cleavage. Catheptic shearing decreases the number of interdigitating linkages causing a reduction in the tensile strength of myofibrils. Miller (1994) reported that several small structural proteins (titin, desmin, and troponin-T) are also influenced by proteolytic activity. Unique to ETC is the apparent

degradation of myosin (Dutson et al. 1977; Yates et al. 1983; Dutson and Pearson 1985) not evident during traditional carcass ageing.

1.11.3.2. Elevated temperature conditioning influence on meat quality

1.11.3.2.1. Tenderness and palatability traits

Smith et al. (1971) applied ETC to beef carcasses (16° versus 2°C for 16 or 20 hours) and observed an improvement in taste panel evaluation of tenderness and a significant reduction in shear force. Parrish et al. (1973) used ETC (16°C versus 2°C for 24 hours) and reported that *Longissimus* steaks from ETC samples were rated more tender and received more desirable scores for flavour and juiciness than conventionally chilled samples. Work by Lee and Ashmore (1985) yielded conflicting results. The experiment involved feedlot (fat) and grass fed (lean) animals with ETC at 35°C for 3 hours compared to conventional chilling at 0°C for 24h. A shorter sarcomere length was observed in lean, conventionally chilled carcasses indicating that cold shortening played a role in tenderness variation. ETC samples, however, were either similar to or tougher than those conventionally chilled and within the fat carcass group, ETC carcasses had significantly shorter sarcomeres than conventionally chilled. It appears that insufficient heat dissipation in well finished carcasses permitted rigor contraction while energy remained available. Jeremiah (1978) concluded that ETC results in very little tenderness improvement, especially when carcasses are aged conventionally following conditioning.

1.11.3.2.2. Appearance scores

ETC has been demonstrated to result in brighter meat colour and enhanced consumer acceptance (Fields et al. 1976) and is useful for the elimination of heat ring, a two-tone colour development resulting from differential chill rates between interior and superficial carcass locations (West 1979). Carcasses with very little insulative fat cover are subject to heat ring development and ETC equilibrates cooling rate, resulting in establishment of uniform colour.

1.11.3.2.3. Concerns with application of elevated temperature conditioning

The primary concern with the use of ETC is the excessive evaporative weight loss associated with holding carcasses at a relatively high temperature (Newbold and Harris 1972; Carse 1973; Bouton et al. 1974). This moisture loss, referred to as cooler shrink, tends to be greater in leaner carcasses (Aalhus et al. 1991) and represents a loss of value from a product that may be evaluated based on weight. Increasing the humidity in holding coolers can counteract shrink loss but provides an advantageous environment for the growth of microorganisms

(Newbold and Harris 1972; Carse 1973; Bouton et al. 1973; Fields et al. 1976). In addition to superficial microbial spoilage, development of off odours at deep locations (bone taint) has been noted as a risk in the application of elevated storage temperature (Jeremiah et al. 1984; Lawrie 1998). While reports of deep spoilage tend to be anecdotal (Joseph and Connolly 1977), bone taint and sour odours can be a concern with the use of ETC.

1.11.4. Blast chilling

Blast chilling is an alternative modification to carcass chilling regime defined by the immediate exposure of a dressed carcass to freezing temperatures (-5 to -70°C) (Aalhus 1995) and rapid air movement (1-5 $m \cdot sec^{-1}$) for 1-5 hours. Following this extreme cold exposure, carcasses are moved to conventional chilling conditions. A rapid chilling regime negates the risk of excessive microbial growth (Marsh 1977) while reducing chilling time, increasing product turnover, and lowering cooler shrink loss (Aalhus et al. 1991). While a rapid cooling rate results in a greater evaporation rate than during slow cooling, this increased moisture loss is offset by a shorter chilling time resulting in lower net shrink loss (Locker et al. 1975). Bowling et al. (1987) reported a significantly lower shrink loss from beef carcasses persisting up to six days postmortem with the use of -70°C chilling for 5 hours as compared to conventional chilling. Aalhus et al. (1994) applied blast chilling to beef carcasses at both -20° and -40°C versus conventional chilling at 2°C and observed that shrink loss was positively related to blast chilling temperature with more rigorous treatments resulting in lower shrink loss.

1.11.4.1. Blast chilling influence on meat quality

1.11.4.1.1. Tenderness and palatability traits

With the use of blast chilling for beef carcasses there is an obvious violation of the "10 in 10 rule" that threatens to negate the advantages of the technology (Aalhus et al. 1991). Martin et al. (1983) stated that rapid cooling rate has a detrimental effect on tenderness, especially during the first 4-5 hours postmortem. The application of blast chilling requires that pre-rigor carcasses be plunged into extreme cold, yielding a pronounced risk of cold shortening. In addition, cold induced toughening may result from the impairment of proteolytic enzyme function. Aalhus et al. (1991) indicated that calpains might be inactivated by cold temperature. Despite these risks, Bowling et al. (1987) successfully applied blast chilling to beef carcasses. According to taste panel assessment, loin steaks from rapidly chilled carcasses showed a significant improvement in tenderness, juiciness and overall palatability.

1.11.4.1.2. Appearance scores

Rapid cooling also influences visually assessed carcass traits. Bowling et al. (1987) reported a significantly higher marbling score in rapidly chilled beef samples owing to the solidification and separation of fat from lean tissue with extreme cold temperature treatment. Typically, meat from blast chilled carcasses is darker and less bright (Bowling et al. 1987; Aalhus et al. 1994) than that from conventional treated carcasses due the effect of cold temperature on the rate and extent of pH decline (Bowling et al. 1987).

1.11.5. Carcass electrical stimulation

1.11.5.1. History of the technology

In 1951, Harsham and Deatherage were granted a patent on a new and unique technology. The team advocated the use of carcass electrical stimulation to promote uniform meat tenderness. The level of tenderness achieved was comparable to that produced by conventional ageing (2-4 weeks) or accelerated ageing (4-5 days at elevated temperature) while consuming much less time and incurring less cost. The traditional ageing process required extensive refrigerated storage space and the costly maintenance of low temperature for an extended period of time. In addition, there was some loss due to trimming areas spoiled during storage and the simultaneous development of off flavours. Accelerated ageing (ETC) required less time and refrigeration input, but cooler shrink losses were high and the temperature/humidity combination provided an environment conducive to microbial growth. The newly proposed technique involved the addition of 10-15 minutes to the carcass dressing procedure, but saved 44 hours or more of cooler time. In 17-36 hours, by the time the carcass was cooled and ready for sale, uniformly tender meat had been produced. Harsham and Deatherage (1951) proposed the mechanism of action to be that of enzyme liberation and activation initiated by the accelerated pH decline in the postmortem tissue. Additionally, a variety of modalities (voltage, amperage, and frequency) were tested, setting the scene for further research. Unfortunately, further investigation did not follow and the technology was all but forgotten until the New Zealand lamb tenderness crisis in the early 1960s. By the early 1970s, work with electrical stimulation in response to this problem had emerged.

New Zealand has long been the largest exporter of lamb and, originally, the distance from markets necessitated the shipment of frozen product (Locker 1985). In the early 1950s pre-rigor blast chilling technology was being used in lamb carcass processing, facilitating an increase in throughput to accommodate an increase in animal production. By 1960 persistent complaints about the toughness of the frozen product were being relayed back to New Zealand. About 80%

of legs and virtually 100% of loins were unacceptably tough (Chrystall and Devine 1985). Freezing was found to be the dominant factor in toughening. The obvious need for a delay prior to freezing was not welcomed by industry; any excess space for hanging carcasses was consumed by the installation of new rapid freezers (Locker et al. 1975). Research efforts were subsequently focused on identifying the toughening mechanism and developing a feasible solution. Both cold- and thaw-shortening were identified as causing toughening and one proposed solution was the use of electrical stimulation.

In a paper representing the first of many on the newly rediscovered technique of carcass electrical stimulation, Carse (1973) demonstrated that electrical stimulation of freshly slaughtered lamb carcasses increased the rate of postmortem glycolysis, in effect hastening the onset of rigor mortis. Acceleration of rigor onset permitted early freezing without the risk of rigor development under temperature conditions conducive to cold shortening. Stimulated carcasses entering the freezer at 5 hours postmortem showed a 46% improvement in objective tenderness over unstimulated samples. Subsequent investigation by this and other workers included a search for the mechanism of action by which electrical stimulation influenced tenderness.

1.11.5.2. Electrical parameters and charge distribution

Examination of the current literature yields a wide array of electrical parameter combinations used in carcass electrical stimulation. These vary in voltage, frequency, amperage, and time (Morton and Newbold 1982; Aalhus 1995). Reports in the literature indicate that voltage and frequency are the most critical in eliciting the desired effects. Voltage is an important consideration in terms of time of stimulation application, charge distribution, and tissue rupture. Higher voltages can be applied later during carcass processing and result in massive contraction so as to cause physical damage to muscle tissue. Frequency is also implicated in tissue rupture depending on the length of the relaxation period between pulses. High frequency combined with high voltage causes sustained contraction and supraphysiological tetanus (Takahashi et al. 1987) resulting in the greatest degree of physical disruption. In general treatments are classified on the basis of voltage (Savell 1985). High voltage electrical stimulation (**HVES**) is generally 550-600 V, 5-15 A, 60 Hz, and 15-20 impulses of 2 seconds each with 1 second between pulses. Low voltage electrical stimulation (**LVES**) is typically 20-90 V, <1 A, 60 Hz, 15-20 seconds total time, delivered in either continuous or pulse form. Each system of electrical parameters is suitable for use under specific conditions (Bouton et al. 1980; Morton and Newbold 1982). The success of either form of electrical stimulation is ensured by using well

rested, unstressed animals (Martin et al. 1983) and by applying the treatment as early as possible in the course of carcass dressing, ensuring adequate substrate for glycolysis.

According to Morton and Newbold (1982), LVES charge is distributed throughout the carcass via the intact nervous system and, therefore, must be applied soon after stunning and before removal of the head from the carcass. HVES is effective by direct cell membrane depolarization with no reliance on the nervous system for charge propagation. For this reason, HVES may be delayed until the carcass has been split, as the voltage is large enough to cause massive contraction of the musculature in the entire carcass or side. Because the voltage involved in HVES is potentially lethal to abattoir workers, elaborate safety precautions must be taken (Aalhus 1995). The combination of safety features required depends on local regulations, but include highly visible warning signs, physical barriers, emergency stop switches, and, while the unit is in operation, flashing lights and audible warning signals (Savell 1985). In addition to safety concerns, there is a sanitation issue accompanying the use of HVES. If the charge is applied prior to evisceration, the massive contraction induced may cause discharge of gastrointestinal tract contents (Savell 1985). For this reason, HVES is generally applied just prior to the carcass being placed in the cooler. Hygiene is also a concern because the probe used to deliver the charge is inserted directly into the *ligamentum nuchae*, in direct contact with neck muscles (Savell 1985; Aalhus 1995). A strict sterilization procedure must be enforced to avoid cross contamination of carcasses. With LVES there is no need for special safety or hygiene procedures because the treatment is applied prior to hide removal, typically via nose clamp, and the voltage is in the non-lethal range (Aalhus 1995). For these reasons, LVES is preferred in a commercial setting (Morton and Newbold 1982; Eikelenboom et al. 1985).

1.11.5.3. Mechanisms of action influencing meat quality

The direct objective in the application of either mode of electrical stimulation is the same. Development of rigor mortis is accelerated by the rapid depletion of energy stores resulting in a concomitant decline in pH (Swatland 1980-81; Aalhus 1995) accompanying the conversion of glycogen to lactic acid (Morton and Newbold 1982). The treatment is considered successful when metabolic events are accelerated both during and after simulation (Morton and Newbold 1982). Low voltage stimulation is equally as effective as HVES in lowering pH (Henrickson and Asghar 1985). Depending on the type of stimulation, however, the ensuing changes in the tissue may vary.

Electrical stimulation initiates metabolic and structural responses in tissue that are directly responsible for changes in meat quality (Henrickson and Asghar 1985). There are four

modes of action by which electrical stimulation can yield an improvement in tenderness (Dutson et al. 1977; Savell et al. 1978; Henrickson and Asghar 1985; Aalhus et al. 1994): prevention of cold shortening, release and activation of autolytic enzymes, tissue rupture, and non-enzymatic weakening of the myofibrillar structure.

1.11.5.3.1. Prevention of cold shortening

Cold acts as a stimulus for contraction (Locker and Hagyard 1963), resulting in shorter sarcomere length and tougher meat (Locker 1960). Cold shortening is a risk where pH is greater than 6.0 (Chrystall and Devine 1985) and where muscle temperature falls below 10°C prior to rigor mortis (Davey et al. 1976). Any ES mode that produces rapid enough pH decline so as to allow chilling within 2 hours is sufficient to prevent cold shortening (Pearson and Dutson 1985). This is the primary reason for the use of LVES. Electrical stimulation accelerates postmortem metabolism such that rigor mortis develops while carcass temperature is still relatively high. Before the carcass temperature declines into the cold shortening range, rigor mortis is fully developed. While calcium remains available as a permissive factor, there is no available energy to fuel contraction (Aalhus 1995). Eikelenboom and Smulders (1986) experimented with LVES using small, lean veal carcasses, a carcass type at risk of cold shortening. A significantly shorter sarcomere length in control samples indicated that cold shortening occurred. The LVES treatment, by preventing cold shortening, yielded a significant improvement in shear value.

1.11.5.3.2. Activation of autolytic enzymes

The issue of postmortem enzyme release and activation and the contribution to tenderization is hotly debated. In their original patent for the electrical stimulation procedure, Harsham and Deatherage (1951) implicated cathepsins (lysosomal proteases) in the tenderization process. They suggested that electrical stimulation not only produced an acidic environment favourable for enzyme activity, but that the treatment somehow made the tissue more susceptible to enzymatic degradation. The theory has subsequently been refined and there are differing schools of thought about the contribution of endogenous proteolytic enzymes to meat tenderness. Marsh et al. (1987) recommended the abandonment of the lysosomal enzyme theory as a factor in electrical stimulation induced tenderization. Ho et al. (1996) suggest proteolysis may contribute to tenderization, but only as a secondary mechanism to physical tissue disruption. Dutson et al. (1980) and Pearson and Dutson (1985) firmly support the low pH, high temperature theory of cathepsin release and activation. Others advocate enzymatic tenderization by the activity of the calpains (Aalhus et al. 1994; Dransfield 1994; Koohmaraie 1996) while early postmortem

conditions favour their activity. Currently, information is still being gathered. While some details are known, the function of the proteolytic systems remains primarily theoretical as their activities prove difficult to study *in vivo*.

Dutson et al. (1977) found an increase in lysosomal enzyme activity following electrical stimulation. The acidic conditions induced while relatively high carcass temperature prevailed resulted in the disruption of lysosomal membranes and release of acidic cathepsins into the cytoplasm (Dutson et al. 1980). Once liberated, the free activity of lysosomal enzymes, β-glucuronidase and cathepsin C, was increased (Dutson et al. 1980; Will et al. 1980), operating maximally at pH <5.22 (Faustman 1994). The noted improvement in tenderness without evidence of sarcomere shortening supports the autolytic proteolysis theory associated with low pH at a relatively high early postmortem temperature (Dutson et al. 1977). Calpains, referred to as neutral proteases, are activated by the rapid intracellular increase of calcium following electrical stimulation (Aalhus et al. 1994), functioning optimally while initial pH and temperature are relatively high. The mechanisms for release and activation of each system is logical, yet the issue surround the contribution of either or both to postmortem tenderization is long from being retired.

1.11.5.3.3. Tissue rupture

Savell et al. (1978) observed that HVES beef carcass sides in a paired sides treatment were more tender than unstimulated sides, yet sarcomere length did not vary between treatments demonstrating that no cold shortening had occurred. The difference in tenderness was explained by physical disruption of tissue. Histological examination using electron microscopy revealed both contracture bands and stretched sarcomeres, indicating that compromised structural integrity may have resulted in greater tenderness. Similar results were achieved by Ho et al. (1996). Contraction nodes with sarcomere length only 44% of resting length were found to be adjacent to sarcomere stretched to 128% of rest length. Fractures in the I band region were noted and there appeared to be some degradation of titin and nebulin. Takahashi et al. (1987) compared 2Hz and 60Hz stimulation and demonstrated that pulse frequency plays a key role in physical disruption of muscle tissue. High frequency produced no uniformity in sarcomere length with zones stretched (up to 32% lengthening) and supercontracted (up to 85% shortening), and regions of complete fracture of myofibrillar structure. Mean sarcomere length was shorter in the high frequency treatment, yet these samples were the tenderest. Will et al. (1980) demonstrated morphological and autolytic effects of HVES on *Longissimus*. At 1 hour postmortem (30 minutes post-stimulation) distinct contraction bands and stretched areas were present and sarcomere integrity

was disrupted by some disintegration of the Z lines. By 6 hours postmortem cells appeared swollen, and at 24 hours postmortem connective tissue had a granular appearance, sarcoplasmic reticulum was swollen, membranes and cristae of mitochondia were swollen and ruptured, and there was a breakdown of myofibrils and the myofilaments within. This work is supported by the observations of George et al. (1980) that demonstrated contraction bands, myofibrillar swelling, and sarcomere distortion by 24 hours postmortem following HVES. Marsh et al. (1980-81) adopted the view that accelerated postmortem glycolysis actually causes toughening; however, the tenderization effect of tissue disruption is greater and offsets the smaller toughening effect of rapid pH decline. LVES and HVES differ in terms of the physical changes effected in muscle tissue and the resulting treatment effectiveness. Only the massive carcass contractions induced by HVES cause disruption of tissue (Aalhus 1995).

1.11.5.3.4. Non-enzymatic weakening of myofibrillar structure

Calcium plays a dual role in postmortem muscle. A rise in concentration induces contraction in the presence of energy compounds, and a further increase weakens intra- and interfibrillar structure (Takahashi 1996). This second function is especially of interest following electrical stimulation treatment during which low pH and physical disruption may compromise the integrity of calcium binding organelles. A flood of calcium into the sarcoplasm appears to initiate five events resulting in non-enzymatic weakening of structural proteins (Takahashi 1996). Although the mechanism has not yet been elucidated, there appears to be a pH dependent, calcium induced weakening of the Z disks. Elevated calcium concentration also weakens rigor linkages by altering the myosin/actin interaction at the A-I junction. Paratropomyosin is translocated from the junction to actin filaments, dominating myosin binding sites because of a greater binding affinity. Actomyosin dissociates thereby releasing rigor tension and restoring the length of rigor shorted sarcomeres. The third calcium influenced change is effected in the titin filaments. Filaments are split resulting in destabilization of the sarcomere, a change that is linearly associated with increasing temperature. Also dependent on both calcium concentration and temperature is the fragmentation of nebulin, a change that results in the destabilization of actin organization. Finally, an increase in calcium concentration results in binding of calcium to and the depolymerization of desmin causing weakening of adjacent Z disk association.

1.11.5.4. Electrical stimulation influence on meat quality

In a carcass processed in a typical commercial operation that does not include ES one would expect relatively slow glycolysis and a wide variability in tenderness (Smulders et al.

1990; O'Halloran et al. 1997). When the onset and completion of rigor mortis is accelerated, tenderness is more uniform and more acceptable (Smulders et al. 1990) and this is the underlying premise of carcass electrical stimulation. The literature, however, contains opposing views on the effect of accelerated postmortem glycolysis and rapid pH decline. Zamora et al. (1996) demonstrated a negative correlation between toughness and rate of pH decline. O'Halloran et al. (1997) suggested that slow versus rapid glycolysis may result in less proteolysis. Marsh et al. (1980-81) voiced opposition to the rapid pH decline/improved tenderness argument. This group stated that acceleration of glycolysis without tissue disruption significantly toughens meat and that slower glycolysis promotes tenderness. Marsh et al. (1987) reported that an intermediate rate of glycolysis resulting in pH near 6.1 at 3 hours postmortem promoted the greatest tenderness.

Successful carcass electrical stimulation influences a range of meat quality attributes including both objectively and subjectively rated tenderness, organoleptic acceptability, lean colour and heat ring, and grade and marbling scores (Pearson and Dutson 1985; Smith 1985; Aalhus 1995).

1.11.5.4.1. Tenderness and palatability traits

The benefit of electrical stimulation for tenderness improvement has been demonstrated repeatedly in the literature. Using HVES, Martin et al. (1983) showed a 16-18% decrease in shear force ($P<0.01$) and Jones et al. (1992) reported a significant ($P<0.001$) 21% improvement in objective tenderness. Aalhus et al. (1992) demonstrated a significantly ($P<0.0001$) lower shear force (5.82 kg versus 7.97 kg) and significantly ($P<0.05$) better taste panel scores for tenderness, flavour, juiciness, and overall palatability with the use of HVES. In experiments designed to examine the effects of both HVES and LVES, results have generally shown a trend towards greater tenderness improvement with more rigorous stimulation treatment.

Not only is tenderness improved by electrical stimulation, but the consistency of the trait is also increased. Davey et al. (1976) reported that electrical stimulation increased the proportion of acceptable samples from 4% to 67% and ensured effective ageing with 91% of samples in highly acceptable range versus 31% for unstimulated samples after ageing. Prolonged ageing, however, was of no additional benefit as demonstrated by George et al. (1980). HVES improved tenderness consistency by up to 98% with the beneficial effect remaining up to 14 days of ageing. Extending the ageing period to 21 days resulted in a decline to 40% in the improvement of HVES over unstimulated controls.

1.11.5.4.2. Appearance scores

Carcass electrical stimulation promotes meat colour that is lighter, brighter, and redder than meat from unstimulated carcasses (Martin et al. 1983; Eikelenboom et al. 1985; Aalhus et al. 1992; Jones et al. 1992; Aalhus,et al. 1994). Smith (1985) stated that slight cases of dark cutting may be ameliorated. The uneven rate of rigor development encouraged by differential cooling rates throughout the carcass (Smith 1985) can result in a two-tone colour development, termed heat ring, across a given muscle. Electrical stimulation promotes rapid and consistent rigor development and the phenomenon of heat ring is alleviated (Martin et al. 1983). Jeremiah et al. (1985) and Aalhus et al. (1992) reported improved subjective marbling score following carcass treatment with electrical stimulation. Pearson and Dutson (1985) and Smith (1985) explained the marbling improvement as a consequence of easier evaluation of firmer fat set against brighter, redder lean. Carcasses subjected to electrical stimulation may receive a more favourable grade score as a combined result of brighter, more youthful appearing lean with more apparent marbling.

1.12. Practical application of postmortem carcass technologies

Given the disadvantages of ETC, including the undesirable processing delay and the requirement of environmentally controlled facilities (Carse 1973), it is not surprising that the technology has not been widely implemented. While there is a beneficial increase in tenderness, the application of ETC to typically encountered, well finished carcasses may actually yield the opposite results (Smith et al. 1979) due to severe rigor shortening. The process would be best applied to lean carcasses arising from grass fed or cull animals, and to alternative livestock genera, such as bison, that tend to be less well finished than commercial beef stock. Because a faster chill rate results in lower cooler shrink loss (Ortner 1989), the use of blast chilling is desirable and represents a significant savings to the commercial packer (Bowling et al. 1987). The trend towards lean carcasses, however, requires the careful application of excessively cold chilling temperatures. The application of electrical stimulation prior to rapid chilling alleviates the potential for cold shortening and can ensure a tender product (Aalhus 1995). Cooler ageing carcasses or vacuum packaged product for an additional 10-14 days is sufficient to ensure a certain degree of tenderization, however, extended storage time can be costly and reduces product flow efficiency. Again, the application of electrical stimulation can be employed as a useful time saving tool by reducing cooler ageing time (Martin et al. 1983).

1.13. Bison carcass handling and the application of alternative techniques

As with the processing of any type of meat producing animals, bison carcass processing poses unique challenges. The bison industry is still developing, and without an industry standard for live animal production, there is potential for variability in the age and condition of slaughter animals. Furthermore, bison carcasses are typically processed in existing slaughter facilities, resulting in the tendency to handle these carcasses similarly to beef carcasses. The lack of a fixed production and processing system introduces the potential for great variability in eating quality of bison meat. There is a paucity of information available about bison carcass and meat characteristics. Without baseline information on which to rely, the development of a genus specific carcass processing system is seriously hindered.

Previous research on traditional meat animal genera has made available volumes of meat science literature on the application of postmortem carcass technologies, an advantage in the development of a suitable bison processing protocol. The typical lack of thick and uniform subcutaneous fat cover (Hawley 1986; Koch et al. 1995) was identified as an initial challenge in bison carcass processing, and served as a starting point for the investigation of alternative processing techniques specifically suited to bison. Experimentation also provided the opportunity to document baseline muscle tissue and meat characteristics.

The overall objectives of the studies presented herein were to characterize carcass, meat quality, nutritional, and histochemical traits of bison muscle tissue and meat and to examine the impact of elevated temperature conditioning, low voltage electrical stimulation, and blast chilling on bison meat quality parameters.

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CHAPTER 2

Characteristics of bison as a meat producing animal

2.1. Introduction

Plains bison (*Bison bison bison*) is the primary subspecies used for commercial bison meat production in Alberta, with a captive wildlife status applied by Agriculture and Agri-Food Canada to farmed animals (Randall 1998). According to Simba Enterprises Ltd. (1998), Alberta producers farmed 22,782 animals in 1996, 50.36% of the total Canadian bison herd. A review of bison production statistics (Table 2-1) indicates that animal numbers are increasing in the province of Alberta, while the number of animals sent for slaughter and processing has fluctuated (Randall 1998).

Table 2-1: Summary of Alberta bison statistics

	1996	1997	1998
Number of producers	386	n/a	609
Total animals (bulls, cows, calves)	22,782	29,345	35,792
Total slaughter animals (provincial and federal plants)	1687	1725	1443*

*January to September

According to The Tracker (1998), an industry oriented Alberta publication, the Canadian Bison Association has >1200 members while the Alberta Bison Association and Peace Country Bison Association have 441 and 168 members, respectively. Bison production and the development and demand of domestic and export markets are expanding (Simba Enterprises Ltd. 1998), with an industry forecast for increasing bison numbers (Randall 1998). Projected herd growth rates are estimated at ~15-20% per year for 1999-2010 (Simba Enterprises Ltd. 1998). Within Alberta, bison is the most readily accepted alternative livestock meat in the hotel and restaurant industry. In fact, interest in bison meat is currently increasing faster than production rate resulting in supply consistency ranking as one of the top concerns prohibiting regular inclusion of bison on menus of the 44 Alberta restaurants surveyed in 1998 (Simba Enterprises Ltd. 1998). Representatives of operations involved with further processing of meat products have also expressed interest in considering bison products in the future once value-added products and appropriate markets have been identified and when animal production is able to meet export demand (Randall 1998).

It appears that all the "industry players" are poised for action, yet there is a large information void in terms of bison carcass, meat, and nutritional characteristics. Several previous

researchers investigating aspects of bison production (Hawley 1986; Marchello et al. 1989; Koch et al. 1995) have noted this absence of empirical data.

The objective of this study was to gather basic data regarding bison carcass and meat quality characteristics and nutrient content in order to contribute to and enlarge the information base for bison meat production and marketing.

2.2. Materials and Methods

2.2.1. Experimental animals

The following information is provided in order to clarify the description of the live animals used in this study. The description has been divided into two parts. Unless otherwise stated, Chapters 2 and 3 refer to control data combined from both parts while Chapters 4 and 5 involve experimental treatment data from Parts 1 and 2, respectively. Descriptions are provided below and are summarized in Table 2-2 for quick reference. As is common in the case of alternative livestock research, herds maintained by institutions tend to be of limited size. Consequently, research is generally based on animals procured from industry sources. The result is often a diverse complement of research stock with varied nutritional and management history, and with a wide range in age (Marchello et al. 1989). This was the situation during the present study.

Part 1 animals:

- 20 feedlot finished, intact male bison from 1995 calving season (April-June).
- December 1997 and January 1998 slaughter dates with animals being 31-32 months of age at slaughter.
- Animals delivered to AAFC LRC (Agriculture and Agri-Food Canada Lacombe Research Centre) and placed in lairage for 19-40 hours with water and straw available.
- An electrolyte supplement (Schaefer et al. 1997) was also made available to some of the animals both on farm and in lairage but was not consumed by the bison in any appreciable quantity.

Part 2 animals:

- 19 feedlot finished, intact male bison from 1996 calving season.
- May and June 1998 slaughter dates with animals being 24-25 months of age at slaughter.
- Animals were delivered to the AAFC LRC on the morning of slaughter and processing began immediately with lairage time ranging from 0-4 hours with water and straw available.

Table 2-2: Source information for experimental bison

	Part 1	Part 2
Calving season	1995 (N=20)	1996 (N=19)
Age at slaughter	31-32 months	24-25 months
Slaughter date	December 1997, January 1998	May, June 1998
Source farm	Curry (electrolyte treatment)	Grinde, Moore
Transport time	2 hours	2-6 hours
Lairage time	19-40 hours	0-4 hours

2.2.2. Slaughter, carcass dressing, and meat sampling

The 39 intact male bison described above were recruited from three Alberta feedlot operations and transported by truck to the AAFC LRC in Lacombe, Alberta for slaughter and study. Liveweight was measured prior to slaughter and, following stunning using a 54 calibre Black Powder rifle loaded with a 230 grain lead ball, carcasses were exsanguinated and dressed in the typical commercial fashion that included the removal of kidneys and kidney knob and channel fat. As part of the experiments discussed in Chapters 4 and 5, carcasses were subjected to various postmortem treatments and sampling procedures for later evaluations. Immediately following stunning and during the exsanguination period, both pH and temperature of the *Longissimus lumborum* (LL) were measured using an Accumet 1002 pH meter with temperature probe (Fisher Scientific, Edmonton, AB) and Orion Ingold Electrode (Udorf, Switzerland). After carcasses were split sagittally, hot side weights were recorded. Dressing percentage was calculated according to the following equation:

$$\text{Dressing percentage (\%)} = \text{hot carcass weight (kg)} \times 100/\text{liveweight (kg)}$$

Sides were rinsed and railed into commercial coolers maintained at ~2°C. After 24 hours, cold side weights were recorded and cooler shrink loss was calculated according to the following equation:

$$\text{Cooler shrink loss } (g \cdot kg^{-1}) = \{(\text{hot side weight (kg)} - \text{cold side weight (kg)}) \times 1000\}/\text{hot side weight (kg)}$$

Cold sides were ribbed at the 11th/12th rib interface. Following a 15 minute bloom period colour of the lean tissue at the exposed site was objectively evaluated. Using a Chroma Meter II (Minolta Camera Company, Japan), colour was measured at three locations on the exposed surface, being careful to avoid areas of clearly visible connective and adipose tissues. Temperature and pH of the LL posterior to the grade site were also measured at this time. Experienced Lacombe Research Centre staff provided a detailed carcass evaluation that included:

maturity class ranking, muscling score, subjective lean and fat colour description, grade fat measurement, and grade. At 24 hours post-mortem, an approximately 100 g LL sample posterior to the grade site was removed to the lab, and immediately prepared for fibre diameter and sarcomere length measurements. Carcass saleable yield cut-out of Part 1 carcasses (left sides) began with forequarters at 24 hours postmortem, with hindquarters completed the following day. At 48 hours post-mortem full striploins (*Longissimus lumborum* posterior from the grade site to the junction of lumbar and sacral vertebrae) were removed from all sides, vacuum packed in oxygen impermeable bags, and stored at 2°C until further analysis at 6 days postmortem. At 6 days striploins were removed from bags and temperature and pH were recorded. After a 15 minute bloom period, objective colour evaluation was completed. Serial steaks were cut (2.54 cm thickness) and designated for further meat quality analyses. Three steaks were removed for shear force determination; one was prepared for analysis later the same day, while the remaining two were vacuum packaged individually in oxygen impermeable bags and stored until 13 and 20 days postmortem, respectively. A fourth steak was made available for driploss determination. As part of an additional analysis (Chapter 5), three steaks were removed from Part 2 carcasses, vacuum packed in oxygen impermeable bags and frozen (-35°C) for later organoleptic analysis. The remaining sections of striploin samples were triple ground through a 3 mm plate (Biro Model 722 grinder, The Biro® Mfg. Co., Marblehead, Ohio) in preparation for analyses of moisture, fat, and protein contents.

2.2.3. Fibre diameter and sarcomere length

From each fresh meat sample removed at 24 hours postmortem, a 2 g subsample was minced with a scalpel and homogenized (Polytron PT 3100, Brinkman Instruments Ltd., Mississauga, ON) with 20.0 ml of 0.25 M sucrose/20mM EGTA solution in a 50.0 ml polypropylene tube. Homogenates were refrigerated at 4°C until all sample preparation was complete. Fibre diameter and sarcomere length measurements were made using wet mount slides of thoroughly mixed homogentate. Under 400x (fibre diameter) and 1000x oil immersion (sarcomere length) phase contrast magnification (Zeiss Axioskop, West Germany), images were captured using Image Pro® Plus (Version 3.0, Media Cybernetics, Silver Springs, MD). For each of the two measurements made, images of ten different myofibres from each sample were captured. Fibre diameter measurements were made using the mouse driven length measurement function and were recorded in millimetres. Mean fibre diameter of the ten images per sample was calculated and converted to micrometres. Sarcomere lengths were measured using the Fast Fourier Transformation function that analyzed the repeating striated pattern and produced a

length measurement in pixels. At 1000x magnification, 6180 pixels were equivalent to 1mm and sarcomere length in micrometres was calculated according to the following equation and averaged across the 10 images per sample:

Sarcomere length (μm) = (pixels/6180) x 1000

2.2.4. Shear force measurement

Cooking, preparation, and cooking loss determination: On days 6, 13, and 20 postmortem striploin steaks were prepared for shear force measurement. Steaks were removed from bags, patted dry with paper towel, and weighed. Weight was recorded as initial steak weight. A stainless steel temperature probe was inserted into the geometric centre of each steak in order to continually monitor internal steak temperature on a data logger (Hewlett Packard 34970A Data Acquisition Switch Unit, Loveland, Colorado). Steaks were placed on a preheated (200°C) electric grill (Garland ED-30B, Garland Commercial Ranges Ltd., Mississauga, ON), cooked to an internal temperature of 40°C, turned, and cooked until a final internal temperature of 72°C was reached. When the final cooking temperature was reached, temperature probes were removed, and steaks were immediately removed from the grill, placed in individual zipper lock bags, and plunged into an ice bath to arrest cooking. With cooking completed, all samples were refrigerated (~4°C) overnight. The following morning, steaks were removed from refrigeration and each was weighed (final steak weight) after patting dry with paper towel. Cooking loss was determined according to the following equation:

Cooking loss (g) = initial steak weight (g) - final steak weight (g)

Shear force measurement: From each steak, four to six 1.9 cm cylindrical cores were removed with a stainless steel corer. Cores were removed parallel to the axis of the grain of the meat so as to allow shearing across the fibres. Cores were placed in a Warner-Bratzler shear cell attached to an Instron Model 4301 Materials Testing System (Burlington, ON). Peak shearing force (kg) was recorded.

2.2.5. Drip loss

Drip loss evaluation steaks were placed on pre-weighed styrofoam trays with dri-loc pads, overwrapped in oxygen permeable film and refrigerated (~4°C) for 10 days. After

refrigeration, steaks were removed from the packaging, patted dry with paper towel and weighed. Drip loss was calculated according to the following equations:

Initial steak weight (g) = (steak + tray) - tray weight

Drip loss ($mg \cdot g^{-1}$) = {(initial steak weight (g) - final steak weight (g)) x 1000}/initial steak weight (g)

2.2.6. Composition analysis

Moisture: Approximately 100 grams of fresh, ground tissue from each carcass side was placed into a pre-weighed (weight a) stainless steel beaker. The ground material was pressed firmly against the inside walls of the containers in order to maximize exposed surface area and ensure even sample drying. Beakers containing wet sample were weighed (weight b) before placing in a convection oven (Precision Scientific Group, GCA Corporation, Chicago, Illinois) for 24 hours at 110°C. When removed from the oven, beakers with dry sample were reweighed (weight c). Moisture content was calculated according to the following equations:

Sample weight (g) = weight b - weight a

Moisture loss (g) = weight b - weight c

Moisture content ($mg \cdot g^{-1}$) = {moisture loss (g) x 1000}/ sample weight (g)

Fat: Dried product obtained after moisture analysis was crushed to a fine powder using a pestle, followed by grinding (Cyclotec 1093 Sample Mill, Tecator, Hoganas, Sweden). Crude fat content was determined using an ether extraction method (Soxtec System HT6, Tecator, Hoganas, Sweden). The standard Soxtec operation procedure was followed using ~5 g dried sample in duplicate weighed in extraction thimbles, 50 ml petroleum ether in aluminum beakers, 10 minutes boiling time, 20 minutes rinsing, 5 minutes ether collection, and 5 minutes ether evaporation. Beakers containing extracted fat were placed in a drying oven at 105°C for 10 minutes before weighing in order to evaporate any residual ether. Crude fat content was calculated according to the following equations:

Sample weight (g) = (thimble + sample weight) - thimble weight

Fat weight (g) = (beaker + fat weight) - beaker weight

Crude fat (mg/g) = {fat (g) x 1000}/sample (g)

Protein: Crude protein of samples dried for moisture content analysis was determined using the FP-428 Nitrogen Determinator (Leco® Corporation, St. Joseph, Michigan). Duplicate samples of approximately 100 mg were weighed into foil cones and tightly wrapped. The

Determinator was standardized with EDTA (ethylenediaminetetraacetic acid) and THAM (Tris hydroxymethyl aminomethane), compounds of known nitrogen content, in order to create a calibration curve. Prepared samples were placed in a carousel which individually placed them into the loading chamber. Under vacuum, the chamber, gas lines and collection ballast were purged of atmospheric gases. Each foil-encapsulated sample was dropped into a furnace (850°C) and flushed with oxygen for rapid combustion. The products of combustion (carbon dioxide, water, dinitrogen, and nitrogen oxides) were passed through a cooler to remove most of the water. A 10 ml aliquot of the combustion mixture was then removed from the collection ballast and passed through hot copper to remove oxygen and convert nitrogen oxides to dinitrogen. Passing the aliquot through Lecosorb® and Anhydrone® removed carbon dioxide and water, respectively. The remaining combustion product, dinitrogen, was measured in the thermal conductivity cell.

The thermal conductivity cell detected differences in the thermal conductivity of gases. Two filaments, the reference and the measure filaments, were maintained under identical gas flow and temperature conditions. Only the composition of the gas flowing through the measure chamber was permitted to change. The reference filament was exposed to helium while the product of sample combustion entered the measuring chamber displacing the normal flow of helium. An increase in temperature of the measure filament was noted due to the lower thermal conductivity of dinitrogen compared to helium. The magnitude of the conductivity difference between the two filaments represented the nitrogen content of the sample. Plotting this difference (represented by the area under a bell-shaped curve) against percent nitrogen in the known standards created a calibration curve. Nitrogen content of samples was estimated from the calibration curve and converted to percent crude protein by multiplying by the typical 6.25 conversion factor.

2.3. Results and discussion

Throughout the following discussion of bison carcass, meat quality, and nutritional traits, comparable beef data sets have been presented in order to serve as recognizable benchmark values. These data sets were chosen on the basis of availability of appropriate information, the use of typical market animals, and similarity of carcass handling procedures, in order to ensure a reasonable comparison.

2.3.1. Carcass traits

Bison carcass data are presented in Table 2-3. Liveweight at slaughter ranged from 429.0 to 507.5 kg with a mean of 472.5 kg and hot carcass weight ranged from 251.2 to 311.6 kg with a mean of 279.2 kg. Accordingly, mean dressing percentage was 59.1% with a range from 55.7% to 61.9%. Hawley (1986) and Koch et al. (1995) reported bison carcass dressing percentages of 59.9% and 62.6%, respectively. Aalhus et al. (1992) reported a dressing percentage range from 58.6% to 61.8% for beef cattle carcasses from animals raised on diverse feeding regimes. Despite the variation in gender, liveweight, and nutritional background, the range of dressing percentages was narrow and for this reason was chosen as representative beef data. The maximum and mean bison dressing percentage values were within the range reported by Aalhus et al. (1992) for beef while the lowest value was below the beef range. In general the bison carcasses tended to have a slightly lower dressing percentage as compared to market beef cattle probably due to the larger head and thicker hide on bison as well as the thick winter hair coat present on the animals slaughtered in December and January. Furthermore, the bison held at the LRC did not pass any gastrointestinal tract contents during the lairage period. It is important to point out, however, that effective comparison of dressing percentage amongst ruminants is difficult because of the variability in volume of rumen contents.

Mean cooler shrink loss from the bison carcasses was 16.0 $g \cdot kg^{-1}$ with a range from 9.8 to 22.5 $g \cdot kg^{-1}$. Aalhus et al. (1992) reported cooler shrink loss values ranging from 11.4 to 16.2 $g \cdot kg^{-1}$ for the same group of beef carcasses discussed above. The tendency for bison carcasses to lose more weight than beef carcasses during conventional cooling is probably due to the greater exposed lean surface area on bison carcasses, from which water is free to evaporate. The distribution of finish on bison carcasses tends to be uneven and localized over the shoulder and loin (Hawley 1986; Koch et al. 1995) resulting in less protection from evaporation for underlying lean tissue as compared to beef carcasses with a more evenly distributed subcutaneous fat cover.

2.3.2. Carcass grade

Current Canadian bison carcass grading standards are presented in Appendix 7.1. Summarizing grade assignment, 12 carcasses graded Canada A1, 16 Canada A2, 1 Canada A3, 3 Canada B1, 4 Canada B2, 2 Canada C1, and 1 Canada D1. Average grade fat thickness was approximately 5.4 mm, with a wide range from <1 mm to 12.5 mm.

Table 2-3: Bison carcass characteristics and *Longissimus lumborum* quality traits

	Mean	SEM	Min	Max
Data from experimental animals*				
Weights (N=20)				
Plant liveweight kg	472.5	4.7	429.0	507.5
Hot weight kg	279.2	3.9	251.2	311.6
Dressing percentage %	59.1	0.4	55.7	61.9
Cold weight kg	274.6	3.7	245.8	305.8
Data from control sides of experimental animals**				
Weight loss (N=20)				
Cooler shrink $g \cdot kg^{-1}$	16.0	1.0	9.8	22.5
Cook loss 6d g	57.09	2.35	40.46	77.37
Cook loss 13d g	61.47	2.75	43.52	84.47
Cook loss 20d g	63.51	4.00	43.91	96.00
Drip loss $mg \cdot g^{-1}$	23.05	1.70	14.89	50.73
Shear kg (N=20)				
6 d	9.55	0.70	5.45	17.99
13 d	7.91	0.75	4.45	18.47
20 d	6.52	0.54	4.01	12.70
pH (N=20)				
0 h	6.83	0.03		
1 h	6.56	0.04		
3 h	6.33	0.07		
10 h	5.88	0.07		
24 h	5.64	0.03		
Temperature C (N=20)				
0 h	38.9	0.2		
1 h	37.8	0.3		
3 h	20.1	0.7		
10 h	3.8	0.3		
24 h	1.5	0.2		
Colour (N=20)				
L* 24 h	30.88	0.39		
Hue_{ab} 24 h	23.51	0.33		
$Chroma_{ab}$ 24 h	21.85	0.60		
L* 6 d	32.11	0.31		
Hue_{ab} 6d	22.57	0.43		
$Chroma_{ab}$ 6 d	22.83	0.54		

*Data from all experimental animals not treated with electrolyte as discussed in Section 2.2.1
**Data combined from untreated sides from the 39 experimental animals discussed in Section 2.2.1

All Canada A1, A2, A3, B1, and B2 grades indicated a youthful carcass class based upon subjective evaluation of the degree of ossification of the cartilaginous caps of the 9th-11th thoracic vertebrae. With the exception of B2, these grades differed only in fat thickness. The Canada B2 grade was assigned where at least one of muscling, fat colour, or lean colour was less than "optimum". In the present study, a limited amount of finish was the most common "defect". The Canada C1 grade was assigned where the carcass was described as being of intermediate maturity. Assignment of a Canada D1 grade was possible in any of the three maturity classes and was used as a "catch-all" classification for various defects in lean and/or fat. The combination of several traits into a single descriptor necessarily results in the loss of detail about any given carcass (Price 1998) and an incomplete flow of information back to live animal producers. The presence of the miscellaneous "D grade" category and the lack of specific "defect" information for individual carcasses demonstrate a weakness of the current grading system. Furthermore, the grading system imposes a hierarchical ranking that implies superiority of one grade over all others (Price 1995). The current carcass grading system is designed to fault carcasses for lack of fat and for excessive ossification at an anatomical point that can be easily altered by an inaccurate sagittal division. More than 25% of the carcasses in the current study would have been ineligible for marketing except as a ground-type, low-value product because of failure to meet "A grade" standards.

Based on the discussion by Price (1998), the implementation of a branded product marketing system for bison would be an ideal method for avoiding the downfalls of the current Canadian bison grading system and for creatively marketing unique products of a guaranteed quality. Asghar and Pearson (1980) stated that there is little association between carcass grade and eating quality, and Crouse et al. (1978) determined that grade accounted for only 2-3% of the variation in taste panel tenderness score and 6-8% of variation in taste panel acceptability. Price (1998) reported that palatability is determined, primarily, by carcass handling in the post-slaughter period and that eating quality is a post-cooking trait only weakly related to carcass measurements. The use of alternative postmortem treatments such as electrical stimulation, altered suspension, or slow chilling is aimed at increasing the level and consistency of tenderness (Fisher et al. 1994) and allows production of tender meat from lean carcasses. Because the bison meat industry is young and free from long-standing consumer traditions and habits, there exists an ideal opportunity to custom design a marketing scheme specifically suited to this new non-beef product.

2.3.3. Carcass yield

Carcass saleable yield and cut-out data are presented in Table 2-4. Saleable yield (weight of saleable meat, trimmed to retail specifications, and calculated as a percent of cold carcass weight) was 78%. Hawley (1986) reported a mean saleable yield of ~77% from 6 bison steers ~2.5 years of age at slaughter with an average liveweight of 444 kg, similar to the bulls used in the present study. The comparative values presented in Table 2-4 demonstrate that carcass saleable yield from the bison was greater than that of the sample population of beef. Koch et al. (1995) reported similar findings upon carcass cut-out comparison. Koch et al. (1995) also reported that bison had less fat trim in all cuts except in the rib section, an area of localized subcutaneous fat deposition.

Also evident was the difference amongst relative weights of individual cuts when bison and beef were compared. As indicated in Table 2-4, the greatest disparity between bison and beef cuts appeared in the forequarter cuts, particularly the blade eye that, for bison carcasses, included the hump. Because of the large dorsal spinous processes, bison carcasses have more meat in the shoulder region than beef (Koch et al. 1995). Berg and Butterfield (1976) reported similar observations; the major difference between bison and beef is in the muscles connecting the neck to the forelimbs, including the hump. The exaggerated size of the forequarter creates the appearance of a disproportionately small hindquarter (Berg and Butterfield 1976; Koch et al.1995), but there was a minimal difference between bison and beef hindquarter cuts.

2.3.4. Meat quality traits

Data representing basic meat quality measures are presented in Table 2-3. Mean shear values at 6, 13, and 20 days postmortem were 9.55, 7.91, and 6.52 kg, respectively. In a controlled comparison of bison and beef, Koch et al. (1995) reported that bison meat had a lower mean shear force value and a greater taste panel acceptability rating for tenderness. It is difficult to compare absolute shear values amongst meat quality studies because of the variety of sampling systems, measurement techniques, and units of measurement. Furthermore, data reported in the literature are generally mean values without the provision of minimum and maximum ranges. The summary (presented in Table 2-5) of mean ranges for studies involving beef cattle of diverse backgrounds (gender, feeding regime, fat thickness) indicates a large variation in shear value.

Table 2-4: Comparison of bison and beef cuts (% side weight) and saleable yield

	Bison (N=20)					Beef* (N=141)			
	Min	**Mean**	**Max**	**SEM**	**Index****	**Min**	**Mean**	**Max**	**SEM**
Forequarter	44.07	45.15	46.47	0.33					
Blade eye (hump)	7.81	9.42	11.03	0.34	250	3.06	3.77	4.66	0.03
Short cut clod	4.22	4.97	5.40	0.11	127	3.21	3.92	4.50	0.02
Chuck tender	1.20	1.27	1.36	0.02	157	0.36	0.81	1.12	0.01
Neck	2.52	3.08	3.68	0.10	102	1.98	3.02	3.87	0.03
Shoulder	1.72	2.22	2.90	0.13	119	1.11	1.87	3.11	0.03
Brisket point	1.99	2.33	2.64	0.06	88	1.95	2.64	3.45	0.03
Short ribs	2.15	2.46	2.97	0.07	262	0.70	0.94	1.21	0.01
Inside skirt (front)	0.39	0.45	0.51	0.01	98	4.67	0.46	7.29	0.01
Foreshank	1.72	1.82	1.94	0.02	113	1.34	1.61	2.29	0.01
Forequarter saleable yield kg	56.60	63.73	69.68	1.26					
Hindquarter	31.30	32.81	33.95	0.31					
Inside round	6.36	6.74	7.10	0.10	119	4.67	5.67	7.29	0.04
Sirloin tip	3.31	3.64	3.90	0.06	124	2.34	2.93	3.61	0.02
Striploin	2.98	3.20	3.43	0.05	102	2.54	3.13	3.83	0.02
Top butt	2.87	3.23	3.46	0.06	100	2.65	3.22	3.84	0.02
Tenderloin	1.97	2.07	2.36	0.04	137	1.02	1.51	1.86	0.01
Flank steak	0.49	0.52	0.61	0.01	104	0.35	0.50	0.63	0.00
Hindquarter saleable yield kg	39.06	46.33	50.9	1.03					
Saleable yield %	75.39	77.96	79.85	0.41	110	65.88	71.15	77.78	0.19

*Canada A1, A2, A3 beef data provided by W. Robertson, Lacombe Research Centre
**Index = (beef mean/bison mean) x 100 (Beef = 100)

Table 2-5: Mean shear value ranges from beef quality studies with various experimental conditions

Study	Conditions	Mean shear value range
Lochner et al. (1980)	• Fat/lean carcasses • -2° or +9°C cooling temperature	6.2-8.8 $kg \cdot cm^{-2}$
Martin et al. (1983)	• Bulls/steers/heifers • Microwave/oven cooking	6.12-9.29 kg
Aalhus et al. (1992)	• Steers/heifers • Variety of finishing regimes	6.27-7.70 kg
Jeremiah et al. (1997)	• Steers/heifers • Canada A1, A2, B1, B2 grades	6.10-12.39 kg

A wide spread in shear values is represented amongst the bison data from the present study suggesting that unless postmortem carcass treatment is appropriate for carcass type, variability of meat tenderness could become a consumer issue for bison meat as is the situation with beef (Aalhus et al. 1992; Marriott and Claus 1994). Solving the problems of variation in tenderness and inconsistency in product quality has been identified as a top priority in the beef industry (Koohmaraie et al. 1996).

According to Jones (1989), the Canadian meat industry has shown a trend towards the processing of leaner carcasses in large, single species abattoirs focused on increased product throughput. The result has been a saving of energy input and labour requirement to chill and trim lean carcasses. At the same time, however, concern has arisen over a decline in meat quality from lean carcasses. Bison carcasses are well suited to the lean carcass production trend, yet if processed according to beef carcass processing standards, they may yield meat of a highly variable quality. Inappropriate processing conditions, especially in the pre-rigor period, can induce toughening in potentially tender meat (Newbold and Harris 1972). It is imperative that processing trends parallel the trend in carcass type in order to ensure appropriate carcass handling techniques are used.

Temperature decline is a function of both ambient conditions and inherent carcass traits such as muscle thickness and fat cover (Forrest et al. 1975; Smith et al. 1976). Compared to conventionally chilled beef carcasses described in Aalhus et al. (1994), the bison carcasses in the present study cooled more rapidly. Aalhus et al. (1994) reported a 3 hour postmortem *Longissimus* muscle temperature range of 27.3-29.5°C and a 24 hour range of 2.6-3.8°C. The LL of bison carcasses in the present study reached mean temperatures of 20.1° and 1.5°C at 3 and 24 hours postmortem, respectively, indicating more rapid cooling. The comparative study was chosen because of the similarity of the carcass cooling procedure to the present study. The difference in cooling rate of bison versus beef may be reflective of the difference in subcutaneous fat distribution between genera.

Decline of pH postmortem, where no other carcass treatments are applied, is a function of muscle temperature (O'Halloran et al. 1997). The temperature of the medium in which glycolytic metabolism occurs directly affects the rate of the enzyme mediated reactions (Pearson and Young 1989). Because postmortem glycolysis is sensitive to slight changes in ambient conditions, a comparison of pH decline between genera is not informative. Ultimate pH, however, is more dependent on the availability of glycolytic substrate at the time of slaughter (Warriss et al. 1989) rather than on temperature (Newbold and Harris 1972). Ultimate pH of the bison meat was within the typical 5.4-5.7 range for red meat (Murray 1995).

Interpretation of objective colour values indicated that at the time of carcass grading the bison meat was darker (lower L^*), more purple red (lower Hue_{ab}), and had a greater colour intensity (higher $Chroma_{ab}$) than the comparable beef samples described in Aalhus et al. (1992). Koch et al. (1995) also reported bison *Longissimus* to be darker than that of beef.

2.3.5. Nutritional quality traits

Results of basic nutrient analysis of bison *Longissimus lumborum* are presented in Table 2-6. The moisture content of 74.9% was very similar to values reported in the literature, 74.5% in comparable bison (Marchello et al. 1989) and 73.6% in Canada A1 beef (Jeremiah et al. 1997). At 21.6%, the present bison protein content was virtually identical to the 21.7% reported by Marchello et al. (1989). These workers also performed a simultaneous analysis of beef. Choice grade beef, similar to Canada AA-AAA available in Canadian grocery stores, contained 21.6% protein, not significantly different from bison, while crude fat content was 7.4%, significantly greater than the 1.9% reported for bison (Marchello et al. 1989). The current result for crude fat content, 1.6%, agrees with this reported value.

For consumers seeking an additional or an alternative meat source, bison may appear attractive as it is commonly marketed with emphasis placed on nutritional qualities to target the health conscious population. The data presented here indicate that bison muscle is lower in fat than beef.

Table 2-6: Analysis of bison *Longissimus lumborum* (N=20)

	Min	Mean	Max	SEM
Moisture content $mg \cdot g^{-1}$	735.9	748.9	765.8	1.9
Crude fat $mg \cdot g^{-1}$ WMB	7.3	16.1	30.9	1.5
Crude protein $mg \cdot g^{-1}$ WMB	202.9	216.4	232.9	2.2

2.4. Conclusion

The bison in this study produced lean carcasses with comparable moisture and protein contents and less fat than a comparable group of beef carcasses. The bison carcasses had a greater yield of marketable meat than beef carcasses. Bison meat tended to be dark, purplish-red. Tenderness was variable, a problem that may be solved with the routine use of postmortem carcass handling techniques designed to influence meat quality in order to produce a consistent product. Implementation of this type of processing would assist with the production and marketing of branded products. Production of bison as a meat producing animal is an expanding industry in Alberta, with growing support for the finished product. Availability of basic information about bison carcass and meat quality will support creative meat processing and marketing techniques, and will enhance receptivity of consumers to this alternative meat product.

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CHAPTER 3

Histological and biochemical characterization of bison muscle tissue and meat

3.1. Introduction

Myofibres are specialized for different types of activity in live muscle (Swatland 1984) and enzymatic and physico-chemical events in postmortem tissue are determined, primarily, by the proportion (Brandstetter et al. 1998) and metabolic properties of distinct fibre types. Many systems of fibre type nomenclature exist, and the three primary types and equivalent terms are summarized as follows: **SO** (Slow Oxidative, red, βR, Type I), **FOG** (Fast Oxidative Glycolytic, intermediate, αR, Type IIA), and **FG** (Fast Glycolytic, white, αW, Type IIB). FG fibres contain an abundance of glycolytic enzymes while SO fibres are predisposed to oxidative metabolism and FOG are intermediate in metabolic characteristics (McCormick 1994).

Knowledge of characteristic postmortem biochemical events is useful when designing appropriate carcass treatments. Variability in response to typical chilling treatments, as well as to alternative treatments such as carcass electrical stimulation, depends on the fibre types present in the tissue and on the availability of metabolic substrate. Examination of fibre dimensions following treatment indicates contraction state (Locker 1960).

The objective of this study was to characterize bison *Longissimus lumborum* (**LL**) in order to provide baseline biochemical and histological data for further studies into postmortem carcass and meat processing.

3.2. Materials and methods

Control data from 39 experimental bison bulls, as described in Section 2.2.1, were used in the present study. Basic slaughter and carcass handling methods were the same as those described in Section 2.2.2 with exceptions as described here. At several times postmortem (0 time (during exsanguination), 1, 3, 10, and 24 hours) small (~50 g) muscle core samples were removed from the LL using a stainless steel corer. Cores were trimmed of subcutaneous fat and connective tissue and immediately placed into liquid nitrogen to halt metabolic activities. Once frozen, cores were bagged (Whirl-Pak) and labeled for storage at -80°C until later assay of glucose, lactate and glycogen. Temperature and pH at the sampling site were also measured at these times using an Accumet 1002 pH meter with temperature probe (Fisher Scientific, Edmonton, AB) and Orion Ingold Electrode (Udorf, Switzerland). At 24 hours postmortem, small (~100 g) LL samples were removed from the control side of each carcass for histological analysis.

3.2.1. Analysis of glycolytic components

Using a method similar to that described in Dalrymple and Hamm (1973) samples were prepared such that glucose, lactate, and glycogen were assayed from a common preparation. Frozen samples were pulverized using a mortar and pestle while maintaining the frozen state with liquid nitrogen. Approximately 1 g of each sample was added to an appropriately labeled 15 ml centrifuge tube along with 5 ml of 0.6 N perchloric acid (to denature proteins allowing release of carbohydrates and metabolites). After homogenization on ice (Polytron PT 3100, Brinkman Instruments Ltd., Mississauga, ON) for 20 seconds at 6000 rpm, a 200 µl aliquot was removed for glycogen determination and added to one tube in an appropriately labeled second set of 15 ml tubes kept on ice. The first set of tubes was centrifuged for 10 minutes at 9000 rpm and 4°C (Beckman J2-MC centrifuge, Palo Alto, CA) before removal of 2.3 ml of supernatant. The supernatant was combined with 200 µl of 3 M potassium carbonate (to neutralize perchloric acid) in a 12x75 mm glass tube and centrifuged for 10 minutes at 3000 rpm and 4°C. These samples were then ready for determination of glucose and lactate levels using a YSI 2300 Stat Plus (YSI Incorporated, Yellow Springs, Ohio) glycogen/lactate analyzer.

For glycogen determination, each tube in the second set received 20µl of 3 M potassium carbonate and 2 ml amyloglucosidase at 14 $\mu g \cdot ml^{-1}$ (to digest glycogen to glucose). Tubes were vortexed, capped, and placed in a 40°C water bath for 2 hours, removing only to vortex every 20 minutes. Following the incubation period, tubes were again placed on ice and 200 µl of 3 N perchloric acid was added to each. Following centrifuging for 10 minutes at 9000 rpm and 4°C, 1.2 ml of supernatant was removed from each tube and placed in 12x75 mm glass tubes with 50 µl 3 M potassium carbonate. After centrifuging for 10 minutes at 3000 rpm and 4°C, samples were ready for glycogen determination (in glucose units) using a YSI 2300 Stat Plus glucose/lactate analyzer.

Sample tubes were loaded into the carousel and, following calibration with known glucose and lactate standards, the YSI analyzer was set to remove duplicate aliquots from each sample tube. The analyzer employed a membrane bound enzymes to catalyze a reaction with the end product (hydrogen peroxide) concentration equivalent to initial carbohydrate content in the sample aliquot. Results were provided in $mmol \cdot l^{-1}$, and converted to $\mu mol \cdot ml^{-1}$ for use in further calculations. Contents of glucose, lactate, and glycogen in assayed samples were calculated according to the following equations:

Glucose and Lactate ($\mu mol \cdot g^{-1}$ tissue) = $\mu mol \cdot ml^{-1}$/(sample weight (g) x 0.16 $g \cdot ml^{-1}$ dilution factor)

Glycogen ($\mu mol \cdot g^{-1}$ tissue) = $\mu mol \cdot ml^{-1}$/(sample weight (g) x 0.0138 $g \cdot ml^{-1}$ dilution factor)

The dilution factors accounted for moisture content of tissue samples and the liquid volumes added and removed during sample preparation.

3.2.2. Histological analysis

Fresh meat samples removed for histological analysis were trimmed into tissue cubes (0.5 x 0.5 x 0.75 cm), cut with the long axis parallel to the fibre grain. The cubes were mounted (Tissue-Tek O.C.T. Compound) on cork (tissue grain perpendicular to cork surface) and quickly frozen in liquid nitrogen prior to freezer storage (-35°C). In preparation for subsequent histological analysis, frozen, mounted samples were thinly sectioned (13 μm) using Tissue-Tek Accu-Edge disposable microtome blades in a Tissue-Tek cryostat unit (Miles Inc. Elkhart, IN) maintained at -20°C. Three to four serial tissue sections were placed onto a room temperature glass slide and allowed to air dry for one hour. Each carcass was represented by two to three slides each bearing three to four sections. Slide mounted tissue samples were stained to make cells readily visible under magnification in order to permit measurement of fibre dimensions and to differentiate three fibre types: **SO** (Slow Oxidative), **FOG** (Fast Oxidative Glycolytic), and **FG** (Fast Glycolytic). The acid preincubation, combined SDH (succinate dehydrogenase)/ATPase staining procedure followed that described in Solomon and Dunn (1988). Acid preincubation destroyed acid labile ATPase in FG and FOG while the SO remained intact. Incubation in the SDH solution highlighted FOG and SO, rich in oxidative enzymes in the mitochondria, with a blue colour while FG remained colourless. Subsequent incubation in the ATPase solution and staining with cobalt chloride produced a brown colour in SO cells, the only fibres with intact ATPase sites. Fibre types were differentiated on the basis of colour development during the staining procedures with the final result showing colourless FG cells, blue rimmed FOG, and brown SO cells. Using image analysis software (Image Pro® Plus Version 3.0, Media Cybernetics, Silver Springs, MD), fibre images were captured under 100x magnification (Zeiss Axioskop, West Germany), counted using an on-screen tagging system and measured using the mouse-controlled measurement function. All cells in each of four fibre bundles from each sample were counted (total 4 bundles per sample) in order to determine the frequency of appearance of each fibre type. For fibre dimensions, the minimum (d) and maximum (D) diameters of 10 of each cell type in three bundles for each sample were examined (total 30 each cell type per

sample). Fibre area was calculated according to the following equation (Clancy and Herlihy 1978):

$$\text{Area } (\mu m^2) = \pi / 4 \{D (\mu m) \times d(\mu m)\}$$

Similar to the work of Seideman and Theer (1986) and Brandstetter et al. (1998) frequency of each fibre type was multiplied by the corresponding fiber area in order to determine percent of total cross-sectional area occupied by each fibre type, and contribution of each type to overall metabolism.

3.2.3. Dentition score classification and statistical analysis

Because of the diversity in research animals involved in the current study, carcasses were categorized on the basis of physiological age in order to isolate any possible effect of age on histological parameters. Dentition score was chosen as the means of approximating physiological age because of the ease of measurement and the relative objectivity of the scoring system as compared to other indicators of physiological age such as ossification of the cartilaginous caps of thoracic vertebral processes (Graham and Price 1982).

Bison have eight teeth, commonly referred to as incisors, across the anterior portion of the lower jaw, four to each side of the midline. A score (maximum possible eight) was assigned to each carcass on the basis of visual examination of dentition with one "point" given for each permanent incisor present. The difference between deciduous and permanent teeth was evident based on size and degree of "wear" on the biting surface (National Buffalo Association 1990). It was assumed that eruption of permanent incisors occurred in pairs. Where only one tooth in a pair had erupted it was assumed that the time interval between teeth in a pair was minimal and the score was raised accordingly. These scores were inserted into the appropriate data sets and run as a class variable in one-way ANOVA analyses using the GLM procedure of SAS (SAS 1990), in order to determine the effect, if any, of physiological age on histological, and biochemical, and quality parameters.

Because of the limited effect of physiological age on fibre area and the lack of effect on frequency and percent total area, pooled figures for each fibre type were used to calculate overall fibre parameter values. These data were compared to beef cattle data derived from typical market animals with histological sections prepared in a similar fashion to that described above. While the beef animals were younger than the bison at slaughter, the comparison represented typical slaughter aged animals for each genus.

3.3. Results and discussion

3.3.1. Postmortem pH decline and glycogen and lactate concentrations

Data for pH decline, and glycogen and lactate concentrations are presented in Figures 3-1 and 3-2 adjacent to data from similarly treated beef carcasses (Bodwell et al. 1965). The bison and beef pH curves demonstrated a faster rate of pH decline with a slightly lower ultimate pH in bison carcasses. Over 24 hours postmortem, mean beef pH declined by 1.25 units while the decline in bison carcasses spanned 1.19 units. From these data, Bodwell et al. (1965) determined that 46.5 $\mu mol \cdot g^{-1}$ lactate was required for each decline of one pH unit in beef *Longissimus*. Similar calculation from the bison data indicated that 62.2 $\mu mol \cdot g^{-1}$ lactate was required per unit of pH decline.

Bendall (1973) demonstrated that initial glycogen content was ~50 $\mu mol \cdot g^{-1}$ for beef *Longissimus* and 55-65 $\mu mol \cdot g^{-1}$ in hog carcasses. Initial lactate concentrations were 16.0 and 6.0-11.2 $\mu mol \cdot g^{-1}$ in beef and pork, respectively. Based on these data and those of Bodwell et al. (1965) it appears that bison LL contained more glycogen than beef and pork at slaughter, and that initial bison LL lactate concentration was similar to beef and greater than that of pork. It is important to appreciate the general limitations of the comparison of relative concentrations of glycolytic substrates and metabolites because these parameter are readily influenced by the antemortem conditions (e.g. nutrition, handling) imposed upon the animals (Warriss et al. 1989).

While the implications of pH decline rate and glycogen and lactate concentrations may not be initially apparent, these data can be useful to those involved in further processing of bison meat products.

Figure 3-1: Comparative pH in postmortem bison and beef *Longissimus*

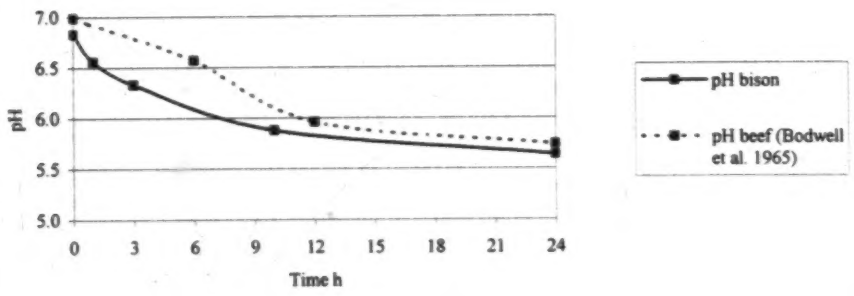
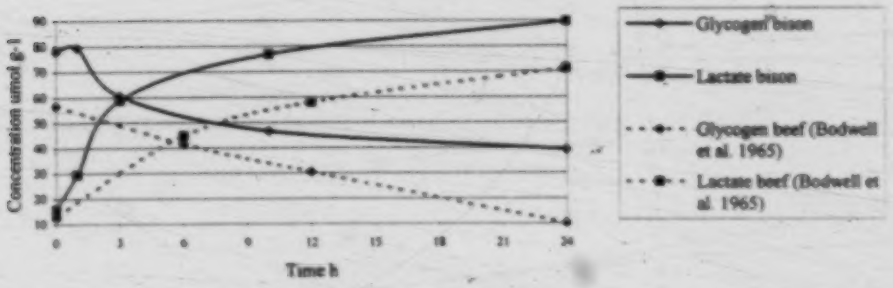


Figure 3-2: Comparative glycogen and lactate concentrations in postmortem bison and beef *Longissimus*



3.3.2. Sarcomere length and fibre diameter

The effect of physiological age on sarcomere length was not significant (Table 3-1). Parrish et al. (1979) also noted a lack of significant maturity class effect on sarcomere length. Because the arrangement of myofibrillar proteins within sarcomeres must be precise in order to allow sychronized muscle contraction, it is logical that sarcomeres would remain of fixed dimensions over time. Muscle growth, then, would occur by the addition of sarcomeres to the length (Swatland 1984) and width of muscle fibres rather than by expansion of individual sarcomeres. Not surprisingly, effect of physiological age was significant for fibre diameter, with older animals having larger fibre diameters. Dumont (1978) explained that while fibre number is fixed at or near birth, fibre diameter increases with age provided that nutrition is adequate so as to allow growth. Within a single animal, however, both sarcomere length (Howard and Judge 1968) and fibre diameter (Swanson et al. 1965) have been observed to vary along the length of a given muscle.

Because the age effect was not significant for sarcomere length, a pooled mean was calculated indicating that conventionally treated bison carcasses yielded a sarcomere length of 1.57±0.05 (SEM) μm at 24 hours postmortem. Amongst a sample of literature reports of sarcomere length for conventionally treated beef *Longissimus* appears a range of mean values from 1.73 to 1.90 μm for sampling times ranging from 24 hours to 14 days postmortem (Hostetler et al. 1972; Joseph and Connolly 1977; Culler et al. 1978; Seideman et al. 1987; Smulders et al. 1990; Whipple et al. 1990). A true measure of initial or resting sarcomere length, however, may never be accurately captured since any measuring technique requires disruption of tissue imposing a stimulus for contraction. Even if a muscle is clamped to preserve its length during removal, localized sections may undergo shortening (Swatland 1984). Comparison between

genera, or even amongst experiments involving similar animals is difficult. Sarcomere length measurement is best incorporated into controlled treatment comparisons and applied as a technique to determine degree of contraction.

Table 3-1: Histological characteristics (SEM) of bison *Longissimus lumborum* (N=18) myofibres

	Dentition Score*			
	0 (N=3)	2 (N=12)	4 (N=5[c],3[d])	P
Sarcomere length μm	1.41 (0.13)	1.60 (0.07)	1.60 (0.10)	0.41
Fibre diameter μm	64.30 (4.72)[a]	82.66 (2.36)[b]	83.70 (3.66)[b]	<0.01
Fibre area μm²				
SO	2491.5 (234.7)[a]	2972.8 (117.3)[a]	3959.2 (234.7)[b]	<0.01
FOG	2965.4 (361.9)[a]	3479.2 (181.0)[a]	4511.4 (361.9)[b]	0.02
FG	5988.4 (855.1)	6465.9 (427.6)	7865.3 (855.1)	0.28
Frequency %				
SO	29.3 (2.8)	28.3 (1.4)	28.7 (2.8)	0.95
FOG	32.3 (3.8)	34.0 (1.9)	31.7 (3.8)	0.83
FG	38.7 (2.3)	37.8 (1.1)	39.7 (2.3)	0.76
% of Total Area				
SO	18.6 (2.6)	19.2 (1.3)	20.2 (2.8)	0.91
FOG	24.1 (3.4)	26.6 (1.7)	25.1 (3.4)	0.78
FG	57.2 (2.8)	54.2 (1.4)	54.6 (2.8)	0.62

*Number of permanent "incisor" teeth as discussed in Section 3.2.3
[a,b] Means in the same row followed by different letters were significantly different
[c] N=5 for sarcomere length and fibre diameter
[d] N=3 for fibre type and fibre area

3.3.3. Myofibre complement and dimensions

Myofibre type frequency, and area data are presented in Table 3-1. The effect of physiological age, as represented by dentition score, was significant only for mean fibre area of SO and FOG fibres. Fibre area tended to increase with age, also reported by Lawrie (1978), however only SO and FOG fibres within dentition score 4 were significantly larger than fibres within scores 0 and 2.

Combined fibre parameter values are presented in Table 3-2 adjacent to comparable beef cattle data. As expected, bison samples displayed a greater mean area than beef for all fibre types. Because fibre area increases with age (Lawrie 1978), these data presumably reflected the difference in age at slaughter between bison and beef animals. The present comparison showed a similar frequency of SO fibre occurrence between genera, while bison had a greater percentage of FOG fibres and lower percentage of FG fibres than cattle. Koch et al. (1995) reported the same trend in fibre type frequency amongst bison and beef cattle. Bison and beef samples had similar percentages of total area occupied by SO fibres, while bison displayed a smaller total area

devoted to FG fibres. Interestingly, despite the lower frequency and smaller total area of FG fibres, bison tissue displayed a more rapid and extensive postmortem metabolism than beef tissue, where 24 hour pH and amount of lactate production were used as metabolic indicators. As compared to beef, however, bison had a larger total area percentage of FOG fibres, and perhaps the large capacity for anaerobic metabolism stems from the glycolytic potential of these fibres.

Table 3-2: Combined bison fibre type values (SEM) and comparative beef data

	Fibre Type		
	SO	**FOG**	**FG**
Bison			
Area μm^2	3057.0 (139.6)	3565.6 (178.5)	6619.6 (357.2)
Frequency %	28.6 (1.1)	33.3 (1.5)	38.3 (0.9)
Percent of total area	19.3 (1.0)	26.0 (1.3)	54.8 (1.1)
Beef: bulls, steer, implanted bulls (Seideman and Theer 1986)			
Area μm^2	2427.0	2695.2	4038.4
Frequency %	26.2	26.6	47.2
Percent of total area	19.8	22.5	57.7
Beef: steers with variable % Bos indicus (Seideman et al. 1987)			
Area μm^2	1859	2120	3595
Frequency %	28.4	23.7	47.8
Percent of total area	19.4	18.5	62.1

Because of different contents of glycogen and metabolic enzymes, histochemical fibre types have varying reactions to the conversion of muscle to meat (Swatland 1984). Muscle fibre complement has been demonstrated to impact upon meat quality traits, although the relationship also depends upon additional environmental conditions. Rahelic and Puac (1980-81) reported that "white" fibres (FG) had a comparatively lower water holding capacity and were tougher than "red" fibres when converted to meat. FG fibres, because of their predisposition to glycolytic metabolism, also display rapid postmortem lactate accumulation (McCormick 1994). The impact of acidification rate on tenderness/toughness is in itself controversial, with literature supporting both slow (Marsh et al. 1980-81) and rapid (Martin et al. 1983) pH decline. The highly developed sarcotubule system within FG fibres enhances the resistance of this fibre type to cold shortening (McCormick 1994), whereas SO fibres have a greater potential to cold shorten (Lawrie 1978; Pearson and Young 1989) due to a comparatively lower capacity to sequester calcium ions (Buege and Marsh 1975). Furthermore, Lawrie (1978) discussed a better tenderization response

with ageing in muscle of predominantly "white" fibres. Thus, knowledge of fibre complement can allow for application of appropriate carcass handling techniques.

In general, farmed bison have been permitted to remain fairly undomesticated and free from artificial selection pressure. A discussion of fibre type complement in relation to domestication introduces convincing arguments for maintaining farmed bison in a relatively "wild" state. Bison carcasses are reported to be virtually free of stress-related meat quality defects, such as DFD (dark, firm, dry), often apparent in beef carcasses (B. Rutley, personal communication 1999). Amongst the 500 carcasses evaluated in Alberta during the development of the current Canadian bison carcass grading system, no cases of DFD were documented (B. Rutley, personal communication 1999). The bison used in the present study appeared calm during transportation and lairage. While the meat tended to be darker than comparable beef samples, none of the carcasses exhibited DFD, as indicated by pH_{24} within the normal range (5.4-5.7, Murray 1995) for red meat.

Intensive livestock production continually exposes animals to conditions different from those to which they have biologically adapted (Ashmore 1974). Generally, in livestock production, there has been increased pressure for faster growing, leaner animals (Ashmore 1974), a trend that has also been identified in meat animal processing (Jones 1989). FOG fibres have the capacity to transform into FG fibres (Ashmore and Doerr 1971), and selective breeding for muscularity has resulted in this general shift and an increased susceptibility to stress in affected animals (Ashmore 1974). In a comparison of wild and domestic pigs, Rahelic and Puac (1980-81) observed a decrease in the percentage of "red" fibres with an increased degree of selection for leanness and muscularity, and that this metabolic shift was accompanied by an increased susceptibility to stress. Natural selection for aerobic muscular endurance amongst wild populations is supported by the need to locate food and water and avoid predators (Ashmore et al. 1972; Solomon et al. 1985). These conditions are altered by intensive production conditions so as to reduce the need for endurance tasks. Infrequent muscle contraction, low energy expenditure, and reduced metabolite exchange requirements are conditions surrounding domesticated animal production and support the shift towards large fibre size and reduced blood flow requirements characteristic of FG fibres. A high plane of nutrition, as supplied in intensive production systems, may also result in a shift from a fibre type that exhibits strong aerobic and ATPase activity (FOG) to those that are predominantly anaerobic with strong ATPase potential (FG) (Swatland 1984). In addition, continual selection of breeding stock based on muscularity would tend to reinforce the accumulation of genetic factors favoring the transformation of FOG to FG fibres (Ashmore et al. 1972). While the proportion of variance in DFD occurrence directly attributable to fibre type is

unknown, the impact of domestication on fibre complement and the apparent absence of the DFD defect in bison carcasses supports the maintenance of a relatively undomesticated production herd, a practice that would reap maximum meat quality potential with minimum labour input.

Given the potential negative impact of a fibre shift towards FG on stress-related meat quality defects, an argument can be made for the use of intact males for meat production. Castration is not currently a typical bison management strategy, and slaughter animals consist mainly of young males. Young and Bass (1984) observed a significantly higher percentage of FG fibres in the *Longissimus* of beef steers versus bulls, while, according to Swatland (1984) heifers had the greatest percentage of FG fibres. The fact that entire males of other meat producing genera may be physiologically reactive to stressors and may exhibit behaviour conducive to stress-related meat quality problems should not be overlooked. Castration is, at present, not an issue in bison production, and it is recommended that producers continue to avoid this procedure in order to maintain a fibre complement with low susceptibility to stress-related meat quality defects.

3.4. Conclusion

Knowledge of the postmortem biochemical nature of bison muscle tissue is informative when designing carcass treatments designed to enhance meat quality. Measures of fibre dimensions can be used as indicators of the impact of such treatments on the contraction state of the tissue. Results from the study of myofibre type complement suggest that bison herds should be maintained as free from production influence as possible in order to maintain the naturally selected and biologically adapted muscle fibre complement that has been successful for the production of carcasses free from stress-related meat quality defects.

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CHAPTER 4

Elevated temperature conditioning of bison carcasses

4.1. Introduction

The rate of carcass cooling in the early postmortem period is a major determinant of meat quality due to its effects on both biochemical and biophysical processes. Inherent carcass characteristics (size, depth of lean and fat) are just as influential as ambient conditions (Lochner et al. 1980) and affect heat exchange and postmortem carcass temperature decline (Aalhus 1995). Carcass cooling rate has a potent influence on glycolytic rate, the resulting rate of pH decline, and proteolytic enzyme activity (Smulders et al. 1990; O'Halloran et al. 1997). Physical configuration of pre-rigor muscle is also affected by cooling rate, contraction state being an important factor in tenderness (Herring et al. 1965). Too early exposure to cold can produce disastrous effects on tenderness (Locker et al. 1975) due to the effects of cold shortening, a major determinant of tenderness in the early pre-rigor period (Smulders et al. 1990) as discussed in Section 1.11.2.

In order to avoid the detrimental effects of cold shortening on tenderness, muscle temperature should be maintained outside the risk range. There is a rule of thumb, commonly referred to as the "10 in 10 rule", stating that to avoid cold shortening muscle temperature should be maintained above 10°C within the first 10 hours postmortem. An obvious means of achieving this condition would be to employ elevated temperature conditioning (**ETC**) by holding dressed carcasses above normal chilling temperature for an appropriate period of time prior to subsequent cooling (West 1979; Marsh et al. 1980-81). This technique is a means of preventing mechanical toughening and promoting biochemical tenderization.

Elevated temperature conditioning is a means of hastening rigor onset (Chrystall and Devine 1985) such that development of rigor is near completion at the time of cold exposure, therefore, lowering the risk of toughening due to cold shortening (also see Sections 1.6.2.and 1.11.3.). Early studies using ETC attributed tenderizing effects solely to this mechanism (Parrish et al. 1969; Smith et al. 1971; Locker 1985). More recently, activity of proteolytic enzymes has been implicated (Parrish et al. 1973; Dutson et al. 1975; Moeller et al. 1976; Dutson et al. 1977; Yates et al. 1983; Dutson and Pearson 1985).

Overall leanness and uneven subcutaneous fat distribution are characteristics of bison carcasses (Hawley 1986; Koch et al. 1995). Because a specific bison-oriented processing system has not yet been developed, commercial carcass cooling conditions have been geared towards beef carcasses that are thicker and have a uniform fat cover. This introduces the risk of "overchill" as defined by Lochner et al. (1980). West (1979) reported that carcasses with thinner

muscling and less fat had the greatest potential for successful ETC application because they typically display a more favourable response to ETC treatment versus thicker carcasses.

The objective of this study was to examine the efficacy of a chilling treatment designed to inhibit potential cold-induced meat quality defects in bison carcasses.

4.2. Materials and methods

4.2.1. Animals, slaughter, postmortem carcass treatment

Twenty bison bulls, as described in Section 2.2.1., were used in the present study. Mean liveweight at slaughter was 496.6±5.57 (SEM) kg with a range from 422.0 to 507.5 kg. Basic slaughter and carcass handling methods were the same as those described in Section 2.2.2. with exceptions as described here. After sides were washed in preparation for chilling, thermocouples were inserted into the *Longissimus lumborum* (loin) and *Semimembranosus* (hip) of each of four paired sides for continuous monitoring of internal muscle temperature during carcass cooling treatments. Temperature data were recorded on a data logger (Hewlett Packard 34970A Data Acquisition Switch Unit, Loveland, Colorado) for 9 hours chilling time (to 10 hours postmortem) and 23 hours chilling time (to 24 hours postmortem) for *Longissimus lumborum* and *Semimembranosus*, respectively. One thermocouple recorded ambient cooler temperature. Alternate left and right sides were moved to conventional (**CONV**) cooler conditions (0-2°C) while the opposite side was exposed to elevated temperature conditioning (**ETC**) (10-12°C) for the first 9 hours of chilling. At 10 hours postmortem, following ETC, treated sides were railed into the conventional cooler for the remainder of the chilling time (to 24 hours postmortem).

4.2.2. Carcass and meat quality analyses

Interval measurements of pH and temperature and core sampling for later analysis of glycolytic metabolites followed the procedure described in Section 3.2.1. Methodologies for the calculation of cooler shrink loss, objective evaluation of colour, and preparation of samples for measurement of fibre diameter, sarcomere length, shear force, cooking loss, and drip loss, were the same as described in Sections 2.2.3., 2.2.4., and 2.2.5.

4.2.3. Statistical analyses

To assess the effects of nutritional treatment, postmortem carcass treatment and their interaction, carcass and meat quality data were analyzed according to a split-plot design using the GLM procedure of SAS (SAS 1990). Electrolyte treatment was in the main plot and carcass

chilling treatment in the sub plot in order to ensure a within animal control for the postmortem carcass treatment.

Model: Y = Diet + Animal(Diet) + Chill + Chill*Diet + Chill*Animal(Diet)
Where: Y = quality parameter
Diet = electrolyte supplement treatment
Chill = carcass chilling treatment

In order to determine and effect of ageing time on objective tenderness measures, time was included in the model for the analysis of shear values over the entire 20 day ageing period.

Model: Y= Diet + Animal(Diet) + Chill + Chill*Diet + Chill* Animal(Diet) + Time + Time*Diet + Time*Animal(Diet) + Time* Chill + Time*Chill*Diet + Time*Chill*Animal(Diet)
Where: Y = quality parameter
Diet = electrolyte supplement treatment
Chill = carcass chilling treatment
Time = total ageing period

In order to assess potential shifts in tenderness over the ageing period and within chilling treatments, a system of tenderness frequency descriptions was used. Ranges in objectively assessed tenderness were divided according to shear values and labeled as follows:

Tender: <5.6 kg; Probably tender: 5.6-<7.85 kg; Probably tough: 7.85-<9.6 kg; Tough: >9.6 kg

Boundaries for tenderness classification categories were based on extensive beef data (Aalhus 1999 unpublished data). Since bison have a similar range in tenderness as beef, frequencies were analyzed by treatment (diet and chill) and within time period (6, 13, 20 days) using the chi-square option of SAS (SAS 1990).

4.3. Results

Some animals received an electrolyte treatment during the ante-mortem period, and, in order to account for this additional live animal variable, the diet treatment was included in the statistical model. No meaningful interactions with chill treatment were noted and this variable has been omitted from further discussion.

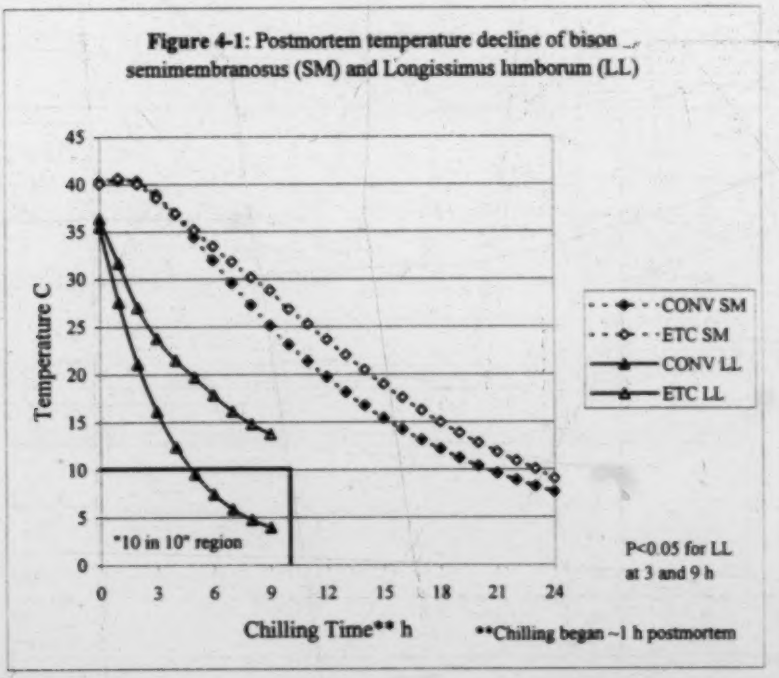
4.3.1. Carcass traits

Mean values for hot carcass weight and dressing percentage were 285.4±3.3 kg and 60.8±0.4%, respectively. Nine carcasses graded Canada A1, 3 Canada A2, 3 Canada B1, 2 Canada B2, 2 Canada C1, and 1 Canada D1.

4.3.2. Temperature decline

Postmortem temperature decline is plotted in Figure 4-1. There was a significant ($P<0.05$) chill treatment effect on *Longissimus lumborum* (LL) temperature decline at 3, 10, and 24 hours postmortem. A more gradual decline in temperature of both LL and *Semimembranosus* (SM) was noted with the use of ETC as compared to CONV chilling. Only CONV LL entered the undesirable temperature zone in which cold shortening is a risk.

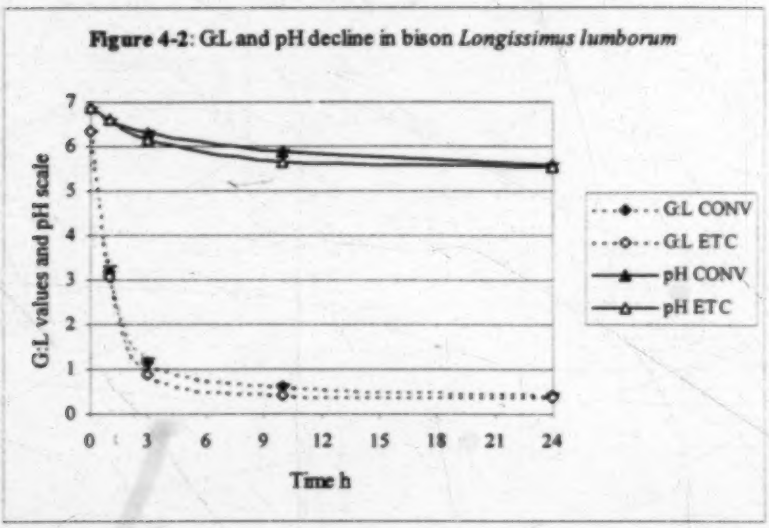
Figure 4-1: Postmortem temperature decline of bison semimembranosus (SM) and Longissimus lumborum (LL)



4.3.3. Biochemical traits

Postmortem pH and glycogen to lactate ratio (G:L) declines are plotted in Figure 4-2. A significant ($P<0.05$) treatment effect on both pH and G:L in *Longissimus lumborum* was apparent at 3, 10, and 24 hour postmortem. ETC resulted in a more rapid decline of both parameters.

Figure 4-2: G:L and pH decline in bison *Longissimus lumborum*



4.3.4. Quality traits

Results for quality traits are presented in Tables 4-1 and 4-2. No measure of carcass or steak weight loss indicated a significant treatment effect (all $P>0.05$). There was a significant ($P<0.01$) treatment effect noted for all objective measures of colour at both 24 hours and 6 days postmortem, except Hue_{ab} at 24 hours ($P>0.05$). Meat from ETC treated carcasses can be described as being lighter and of a more intense cherry-red colour than meat from CONV treated carcasses. There was no significant chill treatment effect on fibre diameter or sarcomere length. Mean shear value of the ETC samples was numerically lower at each test period, significantly so at days 6 ($P=0.02$) and 13 ($P<0.01$).

4.3.5. Tenderness frequency and ageing

A clearly demonstrated trend is evident in the histogram plot (Figure 4-3) of count frequencies in each tenderness category as defined in Section 4.2.3. For both CONV and ETC chilling treatments there was a shift towards a greater number of "tender" samples over time. Comparing the two treatments, however, it was evident that a greater number of "tender" and "probably tender" samples are found within the ETC treatment group. While there was no significant effect of chill treatment on tenderness classes on any given day during the ageing period, the overall effect of time was significant ($P<0.01$) in terms of a reduction in shear force by 20 days postmortem. By breaking down the distribution of samples into tenderness categories the shift from "tough" to "tender" positions on the tenderness continuum is demonstrated within each treatment group over the entire ageing period.

Table 4-1: Bison *Longissimus lumborum* quality traits by chill treatment

	CONV* (N=20)	ETC** (N=20)	SEM	P
Cold weight kg	140.30	139.15	0.87	0.36
Weight loss				
Cooler shrink $g \cdot kg^{-1}$	20.58	21.01	0.42	0.47
Cook loss 6d g	64.35	59.26	3.78	0.35
Cook loss 13d g	68.55	62.66	2.98	0.18
Cook loss 20d g	65.12	70.19	4.00	0.38
Drip loss $mg \cdot g^{-1}$	20.48	21.66	1.07	0.45
Colour				
L* 24 h	30.35	31.83	0.17	<0.01
a* 24 h	20.25	23.18	0.26	<0.01
b* 24 h	8.75	10.04	0.14	<0.01
Hue 24 h	23.36	23.42	0.24	0.87
Chroma 24 h	22.06	25.26	0.28	<0.01
L* 6 d	31.72	32.99	0.15	<0.01
a* 6 d	21.39	22.77	0.18	<0.01
b* 6 d	8.76	9.67	0.11	<0.01
Hue 6 d	22.23	22.97	0.15	<0.01
Chroma 6 d	23.11	24.74	0.20	<0.01
Fibre diameter μm	84.74	82.88	1.35	0.34
Sarcomere length μm	1.63	1.72	0.04	0.15
Shear kg				
6 d	8.22	7.04	0.32	0.02
13 d	7.28	5.68	0.35	<0.01
20 d	5.90	5.13	0.37	0.16

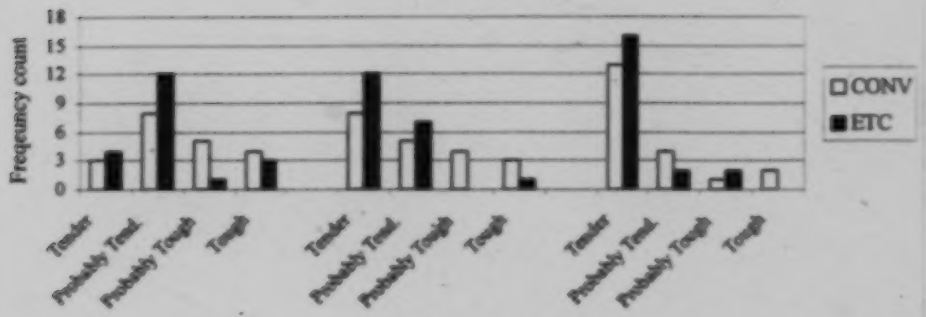
*Conventional chilling treatment
**Elevated temperature conditioning treatment

Table 4-2: Absolute shear value (kg) and sarcomere length (μm) ranges by treatment and ageing time

	Min	Mean	Max
CONV* Shear 6 d	10.44	8.22	17.99
ETC** Shear 6 d	4.32	7.04	13.81
CONV Shear 13 d	5.45	7.28	18.47
ETC Shear 13 d	3.8	5.68	12.38
CONV Shear 20 d	4.14	5.90	12.70
ETC Shear 20 d	3.45	5.13	9.49
CONV Sarcomere length	1.15	1.63	1.84
ETC Sarcomere length	1.27	1.72	1.92

*Conventional chilling treatment
**Elevated temperature conditioning treatment

Figure 4-3: Tenderness frequency counts of bison *Longissimus lumborum* steaks during ageing



4.4. Discussion

4.4.1. Temperature decline

The ETC treatment was sufficient to maintain internal LL temperature above 10°C for the first 10 hours of chilling, an indicator of success of the treatment. From examination of the temperature decline curve of the LL muscle, it was apparent that only CONV LL entered the temperature zone in which cold shortening can be a risk. Mean sarcomere length at 24 hours indicated numerically shorter sarcomeres in CONV versus ETC samples (1.63 vs. 1.72 μm), however, the difference was not significant ($P=0.15$).

During carcass cutout (reported in Section 2.3.3.), an off odour was detected in at least one SM, by an experienced meat technician. Although not quantified in this study, there are similar cases reported in the literature where beef carcasses were slow-chilled by holding at an elevated temperature in the early postmortem period and subsequently developed a sour odour (Joseph and Connolly 1977; Jeremiah et al. 1984). Lawrie (1998) described this type of undesirable odour as bone taint and attributed its presence to microbial growth exacerbated by insufficient cooling of deep-seated locations.

4.4.2. Weight loss

Cooler shrink loss, cook loss, and drip loss were grouped together as weight loss values with the underlying assumption that water was the principle component of any weight change. Moisture can be lost by evaporation from all surfaces of a carcass or by fluid seepage from cut surfaces in the case of steaks. Results demonstrated no significant chilling effect ($P>0.05$) on any

of these parameters. This is of particular practical importance when considering the greater evaporative rate of water from carcasses exposed to ETC versus CONV chilling. Literature results document the risk of moisture weight loss and the necessity for the maintenance of a high ambient relative humidity to counteract evaporative loss when carcasses are held at elevated temperatures (Newbold and Harris 1972; Carse 1973; Bouton et al. 1974). Loss of water from the carcass during cooling represents a loss of saleable product weight. This has been a primary argument against the implementation of elevated temperature conditioning. The present time/temperature conditions may not have been extreme enough to result in a statistically significant treatment effect, but loss of carcass weight is a practical concern and represents an economic loss to a commercial packing operation.

4.4.3. Fibre diameter

No significant chilling treatment effect on fibre diameter was noted as would be expected where cooler shrink loss was also non-significant. Maintenance of fibre diameter is a matter of maintaining intracellular hydration. Fibres that lost only a marginal amount of moisture during chilling could be expected to display only a marginal loss in fibre diameter, as was the present situation. Neither moisture loss nor fibre diameter reduction was of significant or practical importance.

4.4.4. Biochemical traits

As the result of exsanguination of the slaughter animal, circulatory function ceases and along with it, the function of the Cori cycle. The result is an intracellular accumulation of lactate and a concomitant decline of cellular pH as the carcass musculature continues its metabolic function in an anaerobic environment (Swatland 1984; Pearson and Young 1989, Lawrie 1998). Because of their interrelationship in the postmortem period, pH and the components of glycolysis can be dealt with in a single discussion of postmortem muscle metabolism.

Work by Bodwell et al. (1965) and Yambayamba et al. (1996) made use of the sum of primary glycolytic substrates and products, generally referred to as glucidic potential (GP), in relation to ante-mortem animal treatment and ultimate meat pH. In terms of postmortem carcass treatments, this type of measurement does not permit the examination of treatment effect on the reactions within the GP sum total. For this reason, the present results of glycolytic analysis are focused on glycogen to lactate ratio (G:L) as a biochemical indicator of postmortem cellular metabolism. Because of the stoichiometric nature of the reactions involved in the conversion of

glycogen to lactate, the ratio expression allows a comparison of relative glycolysis rates amongst carcasses, without a "clutter" of absolute values.

Given that enzymes of glycolysis function optimally at or near physiological temperatures, it is not surprising that ETC samples exhibited significantly faster glycolytic activity, reflected in the steeper decline of the ETC versus CONV G:L curve, once chilling treatments commenced. The postmortem decline in muscle pH followed a similar pattern as would be expected since the lowering of pH postmortem is directly related to the amount of lactate produced by anaerobic metabolism.

4.4.5. Colour

Any treatment designed to promote rapid postmortem decline of muscle pH will necessarily result in lighter, redder meat because of the preferential binding of oxygen to myoglobin as opposed to its consumption by mitochondria (refer to Appendix 7.5. on colour development). The development of a lighter, more intense red colour with the use of ETC versus CONV chilling is in agreement with literature data. Fields et al. (1976) observed that ETC enhanced visual appearance and consumer acceptance by promoting a brighter colour in treated samples, a reflection of a small but significant difference in pH. West (1979) noted a reduction in colour variability with the use of ETC, but stated that the overall treatment effect was minimal when the elevated temperature employed was less than 30°C. At the time of carcass grading, 24 hours postmortem, the colour objectively assessed in ETC carcasses was preferable as compared to CONV carcasses. Because lean colour is a component of the current bison grading system that rewards bright red lean colour, the ETC treatment could result in a more favourable grade assignment. This significantly brighter, more intense red colour persisted to 6 days postmortem at which time the second and final objective colour assessment was completed. It is reasonable to assume that some product may be marketed at this time and, because consumers prefer the bright red appearance of lean meat, the practical importance of the ETC treatment to colour development is highlighted.

4.4.6. Sarcomere length

While not significant, mean sarcomere length of ETC samples was numerically greater than that of the CONV chill group (1.72 vs. 1.63 μm, P=0.15). It appears that sarcomere shortening during CONV chilling occurred but this observation cannot be attributed solely to the chilling treatment. Examination of minimum and maximum values (Table 4-2) indicates the same trend; sarcomere length of CONV samples was shorter than ETC samples. These extreme

values contribute to the cold shortening argument, indicating practical importance, and suggesting the involvement of an additional chill treatment effect.

When absolute sarcomere lengths are aligned with the corresponding sample shear force values an interesting sarcomere length/toughness story emerges. Amongst the CONV samples, the next to shortest sarcomere length (1.22 μm) was associated with the greatest shear force values at both 6 and 13 days postmortem (17.99 and 18.47 kg, respectively). The minimum sarcomere length (1.15 μm) was associated with shear force values of 9.67, 9.23, and 6.42 kg at 6, 13, and 20 days postmortem. All values are in either the "tough" or "probably tough" tenderness categories (as defined in Section 4.2.3.) and show little positive response to ageing suggesting that extremely short sarcomeres may be resistant to postmortem tenderization. Bouton et al. (1973) reported that ageing could relieve some toughness caused by cold shortening but not if sarcomere contraction has been too severe. Amongst the ETC samples, the minimum sarcomere length was 1.27 μm, similar to the first CONV example presented. The associated shear value, however, demonstrates a radically different picture. At 6, 13, and 20 days, shears values from this sample were 7.43, 5.40,and 4.69 kg, respectively. All values are well within the boundaries of the "probably tender" and "tender" categories despite a relatively short sarcomere length. The maximum sarcomere length resulting from ETC treatment was 1.92 μm with very low associated shear values (6.32, 4.43, 3.63 kg at 6, 13, and 20 days). The maximum sarcomere length arising from CONV treatment was 1.84 μm with associated shear force values comparable to those from the minimum ETC sarcomere length sample.

The relationship between sarcomere length and associated shear force value appears to be dependent on chilling treatment. With the use of CONV chilling the relationship is quite direct, shorter sarcomeres equate to greater shear value. With ETC chilling this simple relationship breaks down because of an additional influence on tenderization. It has been suggested (Moeller et al. 1976; Yates et al. 1983) that endogenous proteolytic enzyme systems are released and function optimally at relatively higher cooling temperatures, and the that application of ETC exploits this process producing a rapid and enhanced ageing effect. Moeller et al. (1976) controlled for sarcomere shortening and demonstrated that the low pH promoted by holding carcasses at high temperature resulted in lysosomal membrane disruption and the release and enhanced activity of proteolytic enzymes. Yates et al. (1983) also demonstrated the proteolytic benefit of low pH in the early postmortem period and reported evidence of myosin degradation and cathepsic shearing of rigor linkages not found in normal ageing conditions. The present results are in agreement with this theory and are supported by the enhanced tenderization response of ETC samples during ageing.

4.4.7. Shear force

Simplified mean shear results indicated a beneficial effect of ETC versus CONV in terms of lowering shear force requirement in all time periods. There was improvement in shear of 14%, 22%, and 13% at 6, 13, and 20 days, respectively, with the use of ETC as compared to CONV carcass chilling. In agreement with literature reports (Parrish et al. 1973; Hostetler et al. 1975; Smith et al. 1979), this reduction in shear force was observed in the absence of a significant treatment effect on sarcomere length.

Examination of minimum and maximum shear values (Table 4-2) provides more information on the range of absolute values encountered in bison loin steaks. The same trend holds true in that ETC samples always had a lower shear value than CONV samples. What is interesting is the extreme range in values and their relationship to the tenderness classification scale set out in Section 4.2.3. The application of CONV carcass chilling produced an initial minimum shear value of 10.44 kg, well within the "tough" category, while the maximum shear value reached as high as 18.47 kg at 13 days. The maximum value encountered in the ETC treatment was 13.81 kg (at 6 days) while the minimum values in all time periods were well within the "tender" category (<5.6 kg). The gap between minimum and maximum values within the ETC treatment was reduced as ageing proceeded (9.49, 8.58, 6.04 kg), while the gap between minimum and maximum fluctuated over time after CONV treatment (7.55, 13.02, 8.56 kg). Using this observation as an indicator of consistency of product response to chilling treatment, ETC is recommended for achieving a uniform tenderness improvement with ageing.

4.4.8. Tenderness frequency and ageing

Examination of the shift in sample numbers amongst tenderness categories provides even more insight into shear value ranges and the influence of postmortem ageing. While the treatment effect on shear value was not statistically significant in any given time period in this analysis, the overall effect of ageing time on tenderization was highly significant ($P<0.01$). Plotting tenderness frequency counts by treatment clearly illustrates the shift towards increased tenderness in the postmortem period, a shift accelerated by ETC treatment, and demonstrates the practical importance of the treatment for reducing postmortem ageing time while maintaining product tenderness.

At 6 and 13 days postmortem there was always a greater count in the "tender" and "probably tender" categories and lesser count in "tough" and "probably tough" for ETC treatment as compared to the counts for CONV treatment. By day 20 this still holds true if the "tender" and "probably tender" categories are combined and "tough" and "probably tough" are combined.

Presumably the meat from the CONV treated carcasses has begun to "catch up" in terms of postmortem tenderization, a phenomenon that was accelerated by ETC. Therefore, combination of the categories was necessary because the finer distinctions between categories readily observable at days 6 and 13 were no longer as clearly defined. There was a shift of one sample from the "probably tender" to the "probably" tough category in the ETC group, however, this shift was offset by the large influx towards the tender category: five samples moved towards "tender" while only one moved towards "tough". At day 20 the original observation of more samples in the "tender" category for the ETC group versus the CONV group remained. By this time no "tough" ETC samples remained, only two were found in each of the "probably tough" and "probably tender" categories, and the remainder were in the "tender" category.

4.5. Conclusion

Elevated temperature was effectively employed as a means of conditioning bison carcasses while avoiding serious cold-induced meat quality defects. The moderate time/temperature combination (10 hours at ~10°C) was not extreme enough to result in significant evaporative loss, although loss of weight during carcass cooling can represent a practical economic loss. In addition, significant improvement in both initial tenderness and tenderization during postmortem ageing was realized with the use of this treatment when compared to CONV carcass chilling. Based on tenderness frequency data, ageing beyond two weeks is not necessary when ETC is used in the immediate postmortem period. Beyond this point, improvement in shear force is minimal while still incurring refrigerated storage costs. The most important practical drawback to the technique is the possibility of off odours developing in deep carcass locations. Due to the unpredictable nature of this type of defect an alternative approach to improving meat quality was explored in a subsequent experiment.

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CHAPTER 5

Blast chilling and low voltage electrical stimulation treatment of bison carcasses

5.1. Introduction

There is a rule of thumb, commonly referred to as the "10 in 10 rule", stating that to avoid cold shortening muscle temperature should be maintained above 10°C within the first 10 hours postmortem (further discussion presented in Sections 1.11.2.2. and 1.11.3.). The most obvious method for achieving this carcass temperature goal is the employment of a cooling regime with an ambient temperature above 10°C, such as with elevated temperature conditioning. A major drawback to the use of any slow cooling technique, however, is the additional cooling time required to complete carcass chilling. Carcass chilling is probably the most costly aspect of meat production in terms of energy expenditure. The use of blast chilling (**BL**) (ambient temperature of -5 to -70° and rapid air movement at 1-5 $m \cdot sec^{-1}$ for 1 to 5 hours) to rapidly cool carcasses dramatically reduces necessary cooler operation time. Additionally, the use of rapid carcass chilling exploits the lower net cooler shrink loss that accompanies a reduced chilling period (Locker et al. 1975; Woltersdorf 1988; Ortner 1989).

Blast chilling of pork carcasses is common commercial practice, and there have been successful applications of the technique for lamb carcasses as demonstrated in the literature (Davey and Garnett 1980; Sheridan 1990). There is evidence that application of BL to beef carcasses may result in cold-induced toughening and compromised meat quality (Aalhus et al. 1991). An impairment of autolytic enzyme systems function may be responsible for toughening (Aalhus et al. 1991) in addition to the expected effects of cold shortening. Based on this evidence, BL may best be used in conjunction with a method designed to accelerate the onset of rigor mortis in order to guard against development of cold shortening.

Electrical stimulation has been demonstrated to improve most aspects of meat quality including tenderness, colour, and palatability attributes (Smith 1985). Low voltage electrical stimulation (**LVES**) has been reported to be less effective for tenderness improvement when compared to high voltage electrical stimulation (Smith 1985), yet LVES application is safer and, hence, a more attractive option (Eikelenboom et al. 1985). The primary mechanism of action for both treatments is the prevention of cold shortening by the acceleration of rigor mortis onset while internal muscle temperature remains outside the cold shortening risk zone. Additionally, high voltage electrical stimulation causes muscular contraction sufficient to cause physical disruption of tissue, a further means of tenderization. Because of its simpler mechanism, LVES is most effective where cold shortening is an actual risk due to low chilling temperatures applied in

the early postmortem period (Marsh et al. 1987), and/or where carcasses are sufficiently lean to result in rapid heat dissipation.

Jones (1989) identified an industry trend towards the production and rapid processing of lean carcasses, citing as a positive benefit a reduction in cost to chill and trim carcasses with less subcutaneous fat. Bison carcasses fit this description with a natural tendency towards minimal finish (Hawley 1986; Koch et al. 1995), but this intrinsic trait introduces a risk for cold-induced meat quality defects when carcasses are processed without regard for leanness.

The objective of this study was to examine the efficacy of a treatment combination designed to rapidly chill bison carcasses while minimizing potential cold-induced meat quality defects by accelerating the onset of rigor mortis.

5.2. Materials and methods

5.2.1. Animals, slaughter, postmortem carcass treatment

Nineteen bison bulls, as described in Section 2.2.1., were used in the present study. Mean liveweight at slaughter was 465.6±5.4 (SEM) kg with a range from 429.0 to 518.0 kg. Basic slaughter and carcass handling methods were the same as those described in Section 2.2.2. with exceptions as described here. A low voltage electrical stimulation (**LVES**) treatment was applied to alternate carcasses using a Jarvis BV-80 unit (Jarvis Products Corporation, Middletown, Connecticut) that delivered an electrical charge (21 volts, 0.25 amps, 60 Hz) via nose clamp for 20 seconds during exsanguination. Untreated carcasses were referred to as **CONTROL**. After sides were washed in preparation for chilling, alternate left and right sides were moved to conventional (**CONV**) cooler conditions (0-2°C) while the paired side was exposed to blast chilling (**BL**) (-20°C, 3 $m \cdot sec^{-1}$ air velocity) for the first 2 hours of cooling. At 3 hours postmortem, following the BL treatment period, treated sides were railed into the conventional cooler for the remainder of the chilling time (to 24 hours postmortem).

5.2.2. Carcass and meat quality analyses

Interval measurements of pH and temperature and core sampling for later analysis of glycolytic metabolites followed the procedure described in Section 3.2.1. Methodologies for the calculation of cooler shrink loss, objective evaluation of colour, and preparation of samples for measurement of fibre diameter, sarcomere length, shear force, and drip loss were the same as described in Sections 2.2.3., 2.2.4., and 2.2.5. As described in Section 2.2.2., additional *Longissimus lumborum* (striploin) steaks were removed at 6 days postmortem, frozen, and stored for later organoleptic analysis.

5.2.3. Organoleptic analysis

Thirty-eight samples (19 carcasses with 2 sides per carcass) were evaluated over the course of six panel sessions, with six or eight samples available at each session. Six experienced panelists were present for each session held in well ventilated, partitioned booths under 1076 lux of incandescent and fluorescent light. Distilled water and unsalted crackers were provided to purge the palate between samples. Stored steaks were removed from the freezer and allowed to thaw at 4°C for 24 hours. A thermocouple was inserted into the geometric centre of each steak prior to cooking on an electric grill (Garland ED-30B, Garland Commercial Ranges Ltd., Mississauga, ON) preheated to 210°C. Steaks were cooked to an internal temperature of 40°C, turned, cooked until internal temperature reached 72°C, and removed from the grill. Following a five minute cooling period, six 1.3cm^3 sub-samples were cut from each steak, colour coded, and placed in a 70°C water bath for 7 minutes to allow for sample temperature equilibration. Each sub-sample was evaluated on a 9-point scale for the following characteristics (Table 5-1):

Table 5-1: Scoring descriptors for organoleptic analysis characteristics

Characteristic	Extreme Scores	
	9	1
Initial tenderness	Extremely tender	Extremely tough
Juiciness	Extremely juicy	Extremely dry
Flavour	Extremely desirable	Extremely undesirable
Flavour intensity	Extremely intense	Extremely bland
Amount of connective tissue	None	Abundant
Overall tenderness	Extremely tender	Extremely tough
Overall palatability	Extremely desirable	Extremely undesirable

5.2.4. Statistical analyses

To assess the effects of postmortem carcass treatments and their interaction, carcass and meat quality and organoleptic analysis data were analyzed according to a split-plot design using the GLM procedure of SAS (SAS 1990). Electrical stimulation treatment was in the main plot and carcass chilling treatment in the sub plot in order to ensure a within animal control for the chilling treatment.

Model: $Y = Stim + Animal(Stim) + Chill + Chill*Stim + Chill*Animal(Stim)$

Where: Y = quality parameter

Stim = electrical stimulation treatment

Chill = carcass chilling treatment

In order to determine any effect of ageing time on objective tenderness measures, time was included in the model for the analysis of shear values over the entire 20 day ageing period.

Model: Y= Stim + Animal(Stim) + Chill + Chill*Stim + Chill* Animal(Stim) + Time + Time*Stim + Time*Animal(Stim) + Time* Chill + Time*Chill*Stim + Time*Chill*Animal(Stim)

Where: Y = quality parameter
Stim = electrical stimulation treatment
Chill = carcass chilling treatment
Time = total ageing period

In order to assess potential shifts in tenderness over the ageing period and within chilling treatments, a system of tenderness frequency descriptions with the following categories was used:

Tender: <5.6 kg; Probably tender: 5.6-<7.85 kg; Probably tough: 7.85-<9.6 kg; Tough: >9.6 kg

Description of category boundaries is discussed in Section 4.2.3. Frequencies were analyzed by treatment (stim and chill) and within time period (6, 13, 20 days) using the chi-square option of SAS (SAS 1990).

5.3. Results

5.3.1. Carcass traits

Mean values for hot carcass weight and dressing percentage were 270.5±3.2 kg and 58.1±0.3 %, respectively. Three carcasses graded Canada A1, 13 Canada A2, 1 Canada A3, and 2 Canada B2.

5.3.2. Biochemical traits

Trends in postmortem *Longissimus lumborum* pH and glycogen to lactate ratio (G:L) declines are represented graphically in Figures 5-1 and 5-2, and the details of LVES and BL treatment interactions are presented in Tables 5-2 and 5-3.

The treatment interaction effect on pH was significant ($P<0.05$) at 1, 3, and 10 hours postmortem. The most rapid pH decline was observed in the LVES carcasses. Numerically, the BL treatment yielded the slowest decline, but this was significantly different only from the LVES treatment. Decline of pH in CONTROL and LVES/BL carcasses was intermediate with no significant difference between treatments. Ultimate pH values were similar amongst treatments with means ranging from 5.56 to 5.60 by 6 days postmortem.

A similar treatment effect was noted for G:L with treatment interaction significant ($P<0.05$) post-stimulation and at 1 hour postmortem. The treatments involving LVES resulted in the most rapid G:L decline, while the BL treatment resulted in a slower, less extensive G:L decline. CONTROL samples were observed to have an intermediate G:L decline. End point G:L for all treatments showed no significant difference and ranged from 0.43 to 0.55 by 24 hours postmortem.

Figure 5-1: Postmortem pH decline in bison *Longissimus lumborum* by stimulation and chill treatments

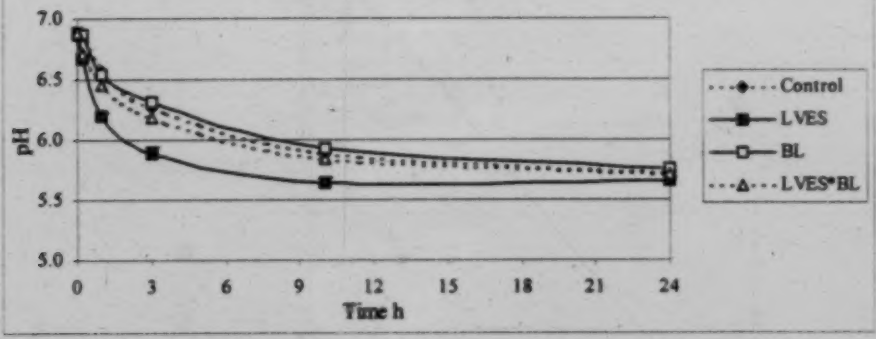


Table 5-2: pH in bison *Longissimus lumborum* at various times postmortem

	CONTROL	LVES*	BL**	LVES/BL***	SEM	P
0 h	6.87	6.89	6.87	6.89	0.00	0.75
PS[y]	6.87	6.67	6.87	6.71	0.02	0.39
1 h	6.57[a]	6.20[b]	6.55[a]	6.45[c]	0.03	<0.01
3 h	6.27[a]	5.88[b]	6.32[a]	6.18[a]	0.06	0.04
10 h	5.88[a]	5.64[b]	5.93[a]	5.85[a]	0.04	0.04
24 h	5.73	5.66	5.76	5.72	0.02	0.38
144 h	5.56	5.58	5.57	5.60	0.02	0.89

*Low voltage electrical stimulation
**Blast chilling
***Combined treatment
[y] Immediately post-stimulation treatment
[a,b,c] Means in the same row followed by different letters were significantly different

Figure 5-2: Postmortem glycogen:lactate (G:L) decline in bison *Longissimus lumborum* by stimulation and chill treatments

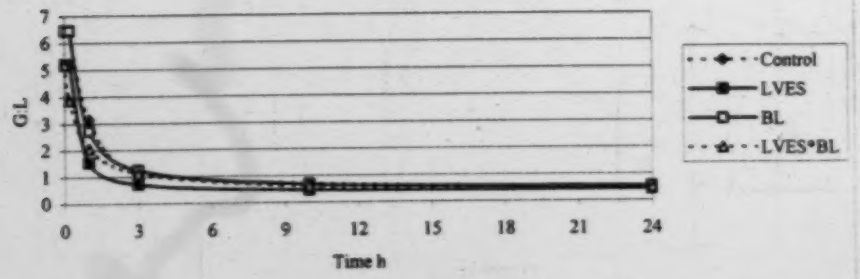


Table 5-3: Glycogen:lactate in bison *Longissimus lumborum* at various times postmortem

	CONTROL	LVES*	BL**	LVES/BL***	SEM	P
0 h	6.47	5.22	6.47	5.22	0.00	0.16
PS[y]	6.47[a]	5.22[b]	6.47[a]	3.89[c]	0.29	0.04
1 h	3.15[a]	1.54[b]	2.73[a]	2.06[c]	0.17	0.01
3 h	1.18	0.72	1.25	1.14	0.11	0.13
10 h	0.71	0.46	0.68	0.6	0.04	0.07
24 h	0.46	0.43	0.55	0.48	0.02	0.20

*Low voltage electrical stimulation
**Blast chilling
***Combined treatment
[y] Immediately Post-stimulation treatment
[a,b,c] Means in the same row followed by different letters were significantly different

5.3.3. Temperature decline

Postmortem temperature decline is plotted in Figure 5-3. There was a significant ($P<0.01$) chill treatment effect on *Longissimus lumborum* (LL) temperature decline at 3, 10, and 24 hours postmortem. The risk of cold shortening development was present for all carcasses as mean internal muscle temperature of the LL in both chilling treatments fell below 10°C within the first 10 hours postmortem. The BL treatment, however, induced this temperature decline more rapidly, with mean LL temperature reaching <10°C by ~3 hours postmortem compared to ~6 hours with the CONV treatment.

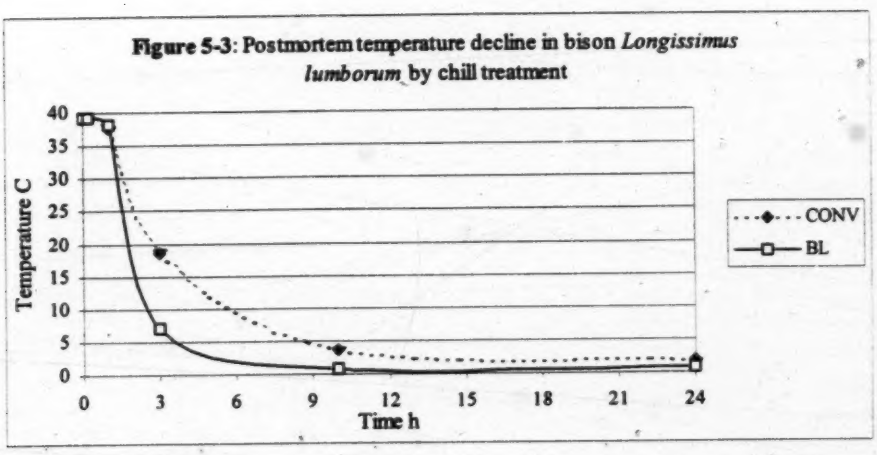


Figure 5-3: Postmortem temperature decline in bison *Longissimus lumborum* by chill treatment

5.3.4. Quality traits

Objective colour data are presented in Table 5-4 and demonstrate simultaneous main effects of both electrical stimulation and blast chilling treatments. Both treatments had significant effects on all objective colour parameters measured at 24 hours postmortem, and the significant effect of LVES on L* persisted until 6 days postmortem. Samples from the LVES treatment could be described as being lighter and of a more intense cherry-red colour while the BL treatment resulted in samples being of a darker, more purple-red colour as compared to CONV.

Results for quality traits are presented in Table 5-5. Both cold carcass side weight and cooler shrink loss were significantly ($P<0.01$) influenced by the chilling treatment. CONV chilled sides weighed less following chilling and, accordingly, had a greater mean cooler shrink loss. Not included in Table 5-5, but important to note, was the lack of significant LVES effect on drip loss. Mean drip loss values were 26.52±2.46 versus 25.10±2.75 $mg \cdot g^{-1}$ for CONTROL and LVES samples, respectively. There was no significant chill treatment effect on fibre diameter while sarcomere length was significantly shorter following BL treatment. Compared to CONV chilling, the BL treatment resulted in greater mean shear values at each test period, significantly so at days 6 ($P<0.05$) and 20 ($P<0.01$).

Table 5-4: Bison *Longissimus lumborum* quality traits by stimulation and chill treatments

	CONTROL (N=10)	LVES* (N=9)	SEM	P LVES	CONV** (N=19)	BL*** (N=19)	SEM	P Chill
Colour 24 h								
L*	30.79	32.59	0.42	<0.01	32.38	31.00	0.24	<0.01
a*	19.40	21.38	0.49	0.01	21.43	19.34	0.32	<0.01
b*	8.10	9.39	0.32	0.01	9.44	8.06	0.19	<0.01
Hue_{ab}	22.60	23.59	0.29	0.03	23.63	22.56	0.14	<0.01
$Chroma_{ab}$	21.03	23.35	0.58	0.01	23.42	20.96	0.37	<0.01
Colour 6 d								
L*	32.44	33.91	0.41	0.02	33.49	32.87	0.22	0.06
a*	20.98	21.87	0.54	0.25	21.75	21.10	0.31	0.15
b*	8.97	9.75	0.44	0.23	9.57	9.15	0.19	0.13
Hue_{ab}	22.99	23.93	0.55	0.24	23.52	23.39	0.17	0.63
$Chroma_{ab}$	22.83	23.95	0.66	0.25	23.78	23.00	0.35	0.14

*Low voltage electrical stimulation treatment
**Conventional chill treatment
***Blast chill treatment

Table 5-5: Bison *Longissimus lumborum* quality traits by chill treatment

	CONV* (N=19)	BL** (N=19)	SEM	P
Shear kg				
6 d	9.86	11.38	0.51	<0.05
13 d	7.12	7.82	0.35	0.17
20 d	6.50	7.52	0.22	<0.01
Fibre diameter μm	85.45	87.46	3.15	0.66
Sarcomere length μm	1.63	1.50	0.03	0.01
Cold weight kg	132.70	134.87	0.44	<0.01
Weight loss				
Cooler shrink $g \cdot kg^{-1}$	15.64	7.28	0.47	<0.01
Drip loss $mg \cdot g^{-1}$	25.74	25.88	1.52	0.95
Cook loss 6d g	52.64	52.57	3.60	0.99
Cook loss 13d g	51.25	48.58	1.21	0.13
Cook loss 20d g	55.25	50.39	2.12	0.12

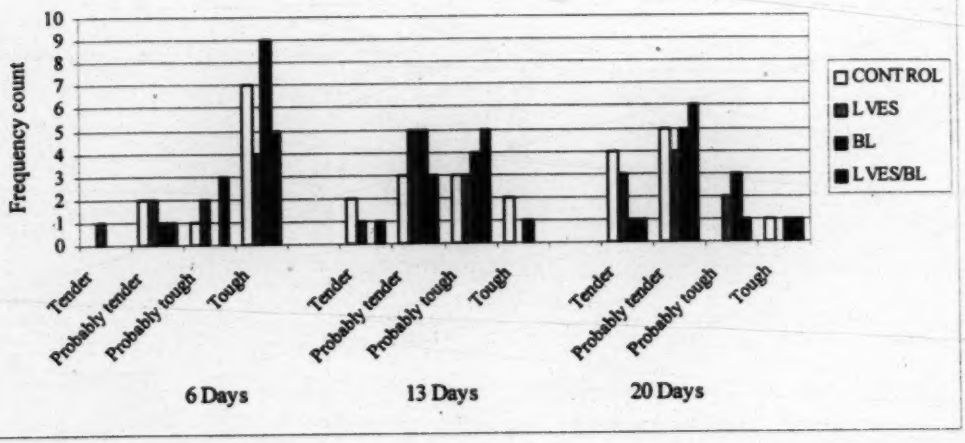
*Conventional chill treatment
**Blast chill treatment

5.3.5. Tenderness frequency and ageing

Figure 5-4 represents the frequency counts across carcass treatments of samples in various tenderness categories (as defined in Section 4.2.3.) over the 20 day ageing period. While

there was no significant treatment effect on frequency count in any given time period, the overall ageing effect was significant ($P<0.01$). When the distribution of samples amongst tenderness categories is plotted across the ageing period, the overall shift towards the "tender" end of the continuum is demonstrated.

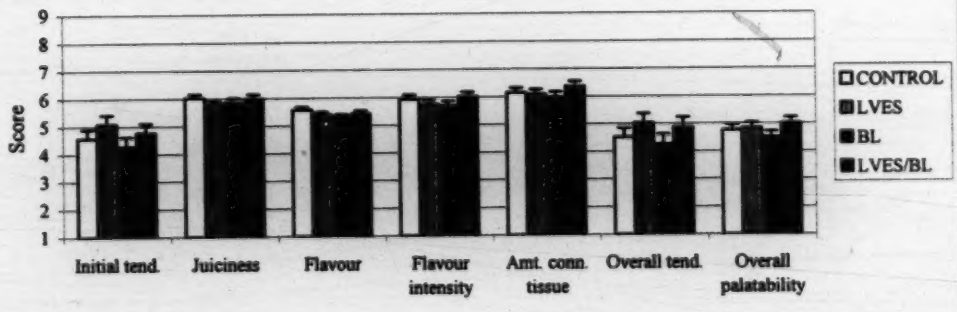
Figure 5-4: Tenderness frequency counts of bison *Longissimus lumborum* steaks during ageing



5.3.6. Organoleptic analysis

Results of organoleptic analysis are presented in Figure 5-5. No significant main effect or interaction was noted in taste panel analysis categories.

Figure 5-5 : Organoleptic analysis of bison *Longissimus lumborum*



5.4. Discussion

5.4.1. Biochemical traits

The rate and extent of postmortem metabolism is dependent on availability of glycolytic substrate at slaughter (Newbold and Harris 1972; Warriss et al. 1989, the temperature of the medium in which the reactions occur (Newbold and Harris 1972; Martin et al. 1983; Pearson and Young 1989; O'Halloran et al. 1997), and whether or not procedures designed to accelerate metabolic reactions have been applied (Carse 1973; Marsh et al. 1987). The absence of the development of meat quality defects such as DFD indicates that the musculature of all carcasses in the present study contained sufficient reserve carbohydrate to sustain normal postmortem anaerobic metabolism. With the intent to prevent cold shortening, LVES is applied early in the carcass dressing procedure as a means of accelerating postmortem glycolytic reactions and hastening the onset of rigor mortis prior to carcass exposure to chilling temperatures. A successful treatment is defined by an accelerated muscle pH decline following stimulation. Because low temperature retards enzyme-mediated reaction rate, as muscle temperature declines, so does the rate of postmortem glycolysis. Unlike LVES, a BL treatment has an inhibitory effect on muscle metabolism and treatment would result in a decelerated pH decline.

The LVES and BL treatments applied in the present study interacted to result in G:L and pH declines representative of the combined treatment. Immediately following LVES, G:L in LVES and LVES/BL sides was significantly lower than in CONTROL and BL. Mean G:L of the LVES/BL samples was significantly lower than in LVES. This value was unexpected since at this time the BL treatment was yet to be applied. The effect was no longer significant at 1 hour postmortem as carcasses entered the coolers. At 3 hours postmortem, following the completion of the BL treatment, LVES sides had a significantly lower G:L compared to all other treatment groups. The inhibitory effect of BL chilling temperature on glycolytic rate balanced the acceleratory effect of LVES in LVES/BL sides. By 10 and 24 hours postmortem no significant treatment interactions remained as G:L values for all treatment groups converged on a similar ultimate value range.

A similar treatment interaction was apparent for pH although the response to treatment seemed to lag slightly as compared to the more immediate changes in G:L. At 1 hour postmortem, pH of LVES and LVES/BL sides was significantly lower than CONTROL and BL. Curiously, at this point in time, LVES/BL samples had a mean pH that was significantly greater than LVES samples despite the fact that BL treatment had not yet begun. Following BL treatment, at 3 and 10 hours postmortem, LVES samples had a significantly lower mean pH than those recorded for all other treatments. As with G:L, the influence of BL chilling temperatures

was to slow pH decline. Measurements at 24 hours and 6 days postmortem demonstrated the convergence of pH towards a common ultimate value.

5.4.2. Temperature decline

The significant chill treatment effect on LL at 3, 10, and 24 hours postmortem indicated that the BL treatment was sufficient to accelerate carcass cooling compared to CONV treatment. Jones (1989) described a meat production industry trend towards species specialized, high volume throughput processing plants. The present results support the use of blast chilling as a routine commercial practice designed to increase production rate by decreasing required chilling time.

This treatment, however, can potentially compromise product quality. The present results indicate: 1) mean LL temperature in both BL and CONV chilled carcasses fell below 10°C within 10 hours postmortem, 2) the significant chill treatment effect on sarcomere length resulted in shorter sarcomere length in BL samples, and 3) BL samples had greater shear force value than CONV samples at 6 ($P<0.05$), 13, and 20 ($P<0.01$) days postmortem. While a causal mechanism linking these observations cannot be determined from the present data, the cold-induced toughening concept that emerges is supported by theories in the literature. Because of its superficial carcass location (Smith et al. 1976), LL temperature is certain to enter the cold shortening risk zone, increasing the risk of shortened sarcomere length and decreased proteolytic enzyme activity (Smith et al. 1976; Aalhus et al. 1991; 1994).

5.4.3. Colour

The LVES treatment resulted in lighter, more intense cherry-red meat colour (refer to Appendix 7.5 for colour development discussion) while the BL treatment resulted in samples that were darker, and more purple-red as compared to CONV at 24 hours postmortem. These results are in agreement with similar studies in the literature.

In an early report on the influence of high voltage electrical stimulation (HVES) on colour, Savell et al. (1978) reported that stimulated sides were brighter than non-stimulated controls. Eikelenboom et al. (1985) and Aalhus et al. (1994) reported similar results for both HVES and LVES treatments. Calkins et al. (1980), however, indicated that the colour enhancing effect of HVES was not persistent when subjectively scored. These results conflict with the objective data of Aalhus et al. (1994) that indicated a persistent effect of HVES to 6 days postmortem. Results of the present study demonstrate that the significant LVES effect on objective brightness (L*) remained until the final objective colour measurement at 6 days

postmortem. Perhaps the subjective evaluation in Calkins et al. (1980) was not refined enough to pick up subtle colour differences beyond 24 hours.

Similar to the present BL results, Bowling et al. (1987) showed that rapidly chilled beef displayed a darker lean colour versus control carcasses due to subtle changes in rate and extent of pH decline during chilling. Aalhus et al. (1991) reported the same effect of blast chilling in beef carcasses, while a combined BL/LVES treatment resulted in samples significantly lighter than conventionally chilled, non-stimulated controls.

Lean colour evaluation is one criterion in the current Canadian bison carcass grading system, with "preferred" grades (Canada A) awarded to carcasses presenting a bright, "youthful" meat colour. Because carcass grading typically occurs at 24 hours postmortem, LVES could be considered a colour enhancing treatment while BL could have a detrimental effect. The use of LVES in conjunction with BL chilling could temper the loss of colour quality resulting from BL alone.

5.4.4. Weight loss

CONV treated sides weighed significantly less than BL sides following chilling and, accordingly, had a significantly greater mean cooler shrink loss confirming statements by Locker et al. (1975) and Ortner (1989) that a faster chilling rate results in a lower net shrink loss. Bowling et al. (1987) reported a persistent and significantly lower shrink loss from beef carcasses with the use of -70°C chilling for 5 hours as compared to conventional chilling. Work by Aalhus et al. (1994) demonstrated that lower cooler shrink loss values followed increasingly rigorous chilling treatment.

As previously noted, there was no significant LVES effect on drip loss. Literature on this topic provides conflicting reports. Eikelenboom et al. (1985) showed that both high and low voltage electrical stimulation significantly increased drip loss. Bouton et al. (1980) reported that HVES steaks were rated less juicy by taste panel members and displayed higher cooking loss. These results support the theory that greater moisture loss accompanied the physical disruption and reduced water holding capacity induced by HVES (Savell et al. 1978). Denaturation of sarcoplasmic proteins resulting from rapid pH decline may also contribute to moisture loss (Eikelenboom and Smulders 1986). George et al. (1980), however, state that increased drip loss was not a risk with the use of electrical stimulation because the sarcolemma takes a long time to become leaky and exudative despite the disruption of sarcoplasmic proteins. This was confirmed by Jones et al. (1992) who reported that HVES produced a significantly lower drip loss versus non-stimulated controls.

5.4.5. Fibre diameter

No significant chill treatment effect on fibre diameter was noted despite the fact that mean cooler shrink loss was significantly lower in BL versus CONV chilled samples. The influences of maintained intracellular hydration and fibre dimension related to sarcomere length reduction may have combined to result in an indirect and non-significant effect of chilling treatment with fibre diameter being larger in BL versus CONV samples.

5.4.6. Sarcomere length

The significantly shorter mean sarcomere length in BL samples was evidence that a certain degree of cold shortening was associated with the extreme temperature treatment of bison carcasses. Based on the theory behind cold shortening and the "10 in 10" rule, this result was not surprising. Smith et al. (1976) stated that muscle would contract to the fullest extent possible when provoked to do so by treatments such as early, rapid carcass chilling. The implication of shortened sarcomere length for tenderness, however, is not as clearly defined as the temperature/shortening relationship. Both Smith et al. (1976) and Lochner et al. (1980) have attributed this imperfect relationship to the temperature dependent involvement of endogenous proteolytic enzymes that may contribute to tenderness/toughness via a process not evidenced in histological measurement of fibre dimensions. Lochner et al. (1980), however, did not rule out the possibility for a more direct cold shortening/toughening relationship in lightweight, lean beef carcasses exposed to rapid chilling early postmortem, a relationship that would seem reasonable for lean bison carcasses with localized finish.

5.4.7. Shear force

Compared to CONV treatment, BL chilling bison carcasses resulted in shear values 15.4%, 9.8%, and 15.7% greater at 6, 13, and 20 days, respectively. Given the significant treatment effect on sarcomere length, it is reasonable to conclude that cold shortening was partly responsible for the increase in shear force for BL samples compared to CONV. Aalhus et al. (1991) found a 6% shear difference in favour of conventional chilling versus blast chilling of beef carcasses; however, applying blast chilling treatments in a later experiment, this group reported numerical but non-significant treatment effects on shear value (Aalhus et al. 1994). In the absence of significant sarcomere shortening, it was suggested that any disparity in shear values might have been the result of impaired activity of endogenous proteolytic enzymes under the extreme cold conditions (Aalhus et al. 1991; Aalhus et al. 1994). In the present study, the greater

difference in shear as compared to results of Aalhus et al. (1991) may reflect additive effects of cold shortening and proteolytic enzyme impairment.

Despite these proposed mechanisms for toughening, earlier work on cryogenic chilling of lamb carcasses (Davey and Garnett 1980; Sheridan 1990) demonstrated the production of tender meat with the use of rapid, early chilling. It was proposed that the development of a frozen, outer surface "crust" was sufficient to restrain muscles from contraction, thereby overriding the effect of cold shortening, and resulting in tender meat. These results are contrary to the results of the present study. Bison carcasses are comparatively larger and "deeper" than lamb carcasses. The conformation of a bison carcass may prevent rapid dissipation of "animal heat" and, combined with the sheer contraction power of large loin and hind limb muscles, the restraining effect of a frozen surface layer would likely be overpowered by the cold shortening mechanism.

5.4.8. Tenderness frequency and ageing

The overall effect of ageing on objective tenderness measurements was significant, representing a reduction in shear force requirement amongst all treatments with increased ageing time. A finer description of the shift amongst tenderness categories and within treatments is demonstrated by plotting frequency counts within tenderness categories as defined by shear value boundaries (as described in Section 5.2.4.). Several interesting points about particular treatments emerged from examination of the tenderness frequency plot.

With 6 days of ageing, only the LVES treatment produced a sample in the "tender" (<5.6 kg) category. In the "tough" category treatments involving LVES had the lowest counts (LVES (4) and LVES/BL (5)) while BL chilling had the highest (9) and CONTROL was intermediate (7). By 13 days all treatments except BL were represented in the "tender" category by at least one sample. Where LVES was incorporated into the treatment, no "tough" samples remained by 13 days. Examining count totals in the "tender" and "probably tender" categories, BL treatment reached a maximum of 6 samples by 20 days postmortem while both treatments involving LVES had totals of 7 and the CONTROL treatment had a count of 9.

Plotting the subtle shifts in tenderness within the overall ageing effect indicates that carcass treatments involving LVES result in sufficient numbers of tender samples by 20 days postmortem to provide a fairly consistent product. BL chilling alone resulted in a lower count in the "tender" and "probably tender" categories, even with 20 days ageing.

Bouton and Harris (1972) claimed that the success of ageing depends on contraction state, and where contracted tissue fails to become more tender during ageing, connective tissue may be involved. Purchas (1972) indicated that tenderness changes during ageing were

associated with the myofibrillar portion of tissue, and not with connective tissue. Evidence suggests that tissue in a contracted configuration may be less amenable to the myofibrillar changes that result in tenderization during ageing. The absolute mechanism for tenderness improvement during the ageing period has yet to be elucidated. Takahashi (1996) summarized the current state of knowledge indicating that while endogenous proteolytic enzymes have been implicated in tenderization during ageing, no work has definitively demonstrated this effect. Furthermore, this author suggested several calcium-mediated, non-enzymatic tenderization mechanisms involving destabilization of myofibrillar protein structure.

It seems apparent that the BL treatment needs to be balanced by a toughness prevention treatment, such as LVES, because of the potential for both cold shortening, as demonstrated in the present results, and impairment of proteolytic enzyme activity suggested in the literature (Aalhus et al. 1991; 1994). Davey et al. (1976) used electrical stimulation of beef carcasses to increase the percentage of acceptable samples by ensuring effective ageing. Aalhus et al. (1992) reported the least improvement with ageing in stimulated versus non-stimulated beef samples; however, it is possible that the treated samples were already sufficiently tender.

The ageing process appears to be somewhat redundant where a toughness prevention treatment has previously been applied. Using HVES, Savell et al. (1981) demonstrated that the maximum benefit of the treatment was achieved with no more than eight days ageing. Furthermore, with the application of LVES aimed at tempering the cold shortening impact of blast chilling, no more than a maximum of 2 weeks ageing should be applied in order to avoid negating the potential for the increased plant throughput that blast chilling treatment offers.

5.4.9. Organoleptic analysis

The present results indicate that no significant treatment effect on taste panel characteristics was detectable. While LVES did not significantly improve organoleptic quality, neither did blast chilling result in a significant loss of quality.

It is interesting to note, however, that when treatments are ranked based on absolute values for the initial and overall tenderness categories, the emerging trend parallels that of absolute shear values at 6 days postmortem:

Lowest shear/Highest tenderness score ⟷ Highest shear/Lowest tenderness score

LVES↔LVES/BL↔CONT↔BL

Literature reports of the effects of both LVES and BL treatments on organoleptic characteristics alternately support and oppose the present data. Aalhus et al. (1994) reported no significant improvement in taste panel scores with the independent applications of LVES and BL. Bowling et al. (1987), however, reported significant improvements in tenderness, juiciness, and overall palatability with the use of a three-phase chilling regime that included a primary 5 hour chill period at -70°C. These results may be misleading, however, because the comparative control treatment referred to as conventional was, at -7°C, outside the typical 0-2°C range typically employed. Additionally, carcasses treated at -70°C were secondarily equilibrated at 16°C for 4 hours. The lower than normal conventional treatment and the high temperature equilibration period for cold treated carcasses may have skewed results in favour of the blast chilling treatment.

5.5. Conclusion

The application of blast chilling to bison carcasses was a successful means of reducing cooler shrink loss and achieving low internal muscle temperature faster than with the use of conventional carcass chilling. From the present results it was obvious that rapid chilling of bison carcasses was not without risk of compromised meat quality due to delayed pH decline and cold shortening of muscle fibres. Blast chilling produced darker meat colour and greater shear values versus conventionally chilled samples. Taste panelists, however, were unable to detect a detrimental chilling treatment effect. The incorporation of the LVES treatment designed to accelerate glycolysis and the onset of rigor mortis tempered the potential negative effects of blast chilling on meat quality. Low voltage electrical stimulation was easy and safe to operate without slowing production line speed, and resulted in lighter coloured meat and a tendency for reduced shear value. In keeping with the identified trend towards increased plant throughput, the combined low voltage electrical stimulation/blast chilling bison carcass treatment is recommended for rapid processing without compromising meat quality.

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Chapter 6

General summary and conclusions

The overall objective of this work was to characterize carcass, meat quality, nutritional, and histochemical traits of bison muscle tissue and meat and to examine the effects of various postmortem carcass handling techniques on meat quality parameters. The studies presented in the previous chapters addressed this goal.

Examination of basic carcass and meat characteristics (refer to **Chapter 2**) indicated that bison carcasses have a greater yield, lower fat, and darker meat colour than comparable beef samples. Moisture and protein contents were similar to beef. Tenderness of bison meat was variable, with a wide range in shear values. There is a need to address the practicality of the current Canadian bison carcass grading system. A streamlined carcass evaluation design would provide useful information to live animal producers enabling the adjustment of management strategies to meet market preferences.

Based on examination of myofibre parameters, indicators of intracellular biochemistry, and the observation that bison carcasses are relatively free of stress-related meat quality defects (refer to **Chapter 3**), commercial production of bison in a relatively undomesticated state is recommended. Bison are well suited to production in the variable climatic conditions of Canada and the biological integrity of this naturally selected genus should be preserved.

Elevated temperature conditioning of bison carcasses (refer to **Chapter 4**) was useful for the production of consistently tender meat with a reduced ageing requirement. The unpredictable risk of spoilage and off odour development, as well as the potential for excessive cooler shrink loss may limit the adoption of this technique in commercial carcass processing operations.

Compared to conventional cooling, blast chilling of bison carcasses decreased cooler shrink loss (refer to **Chapter 5**). Taste panel evaluation of organoleptic characteristics indicated no detectable, detrimental effects of blast chilling. Objective measures of tenderness indicated that low voltage electrical stimulation, and stimulation combined with blast chilling yielded the most tender samples. This combined carcass treatment appears to be a practical alternative to conventional carcass handling and would promote consistent meat quality while allowing for rapid product throughput.

Incorporation, into standard carcass processing, of alternative postmortem carcass handling techniques designed to enhance meat quality, would allow for marketing of branded products with an assured and consistent eating quality.

The work presented here represents an introductory foray into the application of alternative postmortem carcass technologies to bison carcasses and the development of a genus specific processing protocol. Future research might focus on the functionality and exploitation of calcium activated proteases in bison meat tenderness, and a refinement of the role of bison meat in human nutrition.

7. APPENDICES

7.1. Canadian bison carcass grading standards

7.2. Tabulated data from analysis of glycolytic components (Chapter 3)

7.3. Tabulated data from analysis of glycolytic components (Chapter 4)

7.4. Tabulated data from analysis of glycolytic components (Chapter 5)

7.5. Postmortem colour development in meat

7.1. Canadian bison carcass grading standards

Table 7-1: Canadian bison carcass grading criteria

Age/Maturity	Quality Factor	A1	A2	A3	B1	B2	C1	C2	D1	D2
Class I (Youthful) Ossification 50% or less	Muscling	Excellent to Good				Exc/Med			No requirement	
	Fat colour	White to Amber and Firm				Wht/Yel			No requirement	
	Meat colour	Bright red and Firm				Brt/Drk red			No requirement	
	Fat thickness mm	1 to 6	7 to 12	>12	0 to 1	>1			0 to 4	>4
Class II (Intermediate) Ossification 51-80%	Muscling						Excellent to Medium		No requirement	
	Fat colour						No requirement		No requirement	
	Meat colour						No requirement		No requirement	
	Fat thickness mm						1 to 4	>4	0 to 4	>4
Class III (Mature) Ossification over 80%	Muscling								No requirement	
	Fat colour								No requirement	
	Meat colour								No requirement	
	Fat thickness mm								1 to 4	>4

Appendix 7.2. Tabulated data from analysis of glycolytic components (Chapter 3)

Table 7-2: Minimum, mean, and maximum concentrations ($\mu mol \cdot g^{-1}$) of glycogen, lactate, and glucose at various times postmortem in *Longissimus lumborum* of conventionally treated bison carcasses

Time h		Min	Mean	Max	SEM
0	Glycogen	7.78	78.06	114.49	5.21
	Lactate	10.15	15.75	29.56	1.28
	Glucose	0.52	1.02	1.65	0.08
1	Glycogen	46.90	79.56	114.20	4.21
	Lactate	15.03	29.39	53.98	2.09
	Glucose	0.44	1.09	2.39	0.11
3	Glycogen	36.00	60.60	94.50	4.19
	Lactate	28.14	58.80	88.99	3.88
	Glucose	0.46	2.35	4.19	0.25
10	Glycogen	17.94	46.60	82.61	3.77
	Lactate	37.41	76.97	116.34	4.35
	Glucose	1.23	3.69	7.47	0.35
24	Glycogen	17.68	39.23	64.63	3.06
	Lactate	78.29	89.72	108.34	1.89
	Glucose	3.85	5.02	6.45	0.17

Appendix 7.3. Tabulated data from analysis of glycolytic components (Chapter 4)

Table 7-3: Mean concentrations ($\mu mol \cdot g^{-1}$) of glycogen, lactate, and glucose at various times postmortem in *Longissimus lumborum* from bison carcasses chilled conventionally (CONV) and with elevated temperature conditioning (ETC)

Time h		CONV	ETC	SEM	P
0	Glycogen	82.27	82.27	0.00	0.30
	Lactate	15.26	15.26	0.00	0.28
	Glucose	1.03	1.03	0.00	0.29
1	Glycogen	78.05	78.02	1.44	0.99
	Lactate	28.08	28.60	1.17	0.76
	Glucose	1.09	1.14	0.09	0.74
3	Glycogen	60.87	56.27	1.61	0.06
	Lactate	59.80	67.70	1.91	<0.01
	Glucose	2.48	2.96	0.14	0.03
10	Glycogen	47.20	39.15	1.37	<0.01
	Lactate	79.76	92.32	1.80	<0.01
	Glucose	3.92	4.65	0.17	<0.01
24	Glycogen	39.14	36.56	0.52	<0.01
	Lactate	89.70	95.80	0.10	<0.01
	Glucose	5.03	5.44	0.12	0.03

Appendix 7.4. Tabulated data from analysis of glycolytic components (Chapter 5)

Table 7-4: Mean concentrations ($\mu mol \cdot g^{-1}$) of glycogen, lactate, and glucose at various times postmortem in *Longissimus lumborum* from bison carcasses treated conventionally (CONTROL), low voltage electrical stimulation (LVES), blast chilling (BL), or a combination of low voltage electrical stimulation and blast chilling (LVES/BL)

Time h		CONTROL	LVES	BL	LVES/BL	SEM	P
0	Glycogen	80.67	77.88	80.67	77.88	0.00	0.47
	Lactate	13.16	17.09	13.16	17.09	0.00	0.19
	Glucose	1.03	1.43	1.03	1.43	0.00	0.16
PS*	Glycogen	80.67	77.88	80.67	77.09	0.64	0.54
	Lactate	13.16[a]	17.09[b]	13.16[a]	20.98[c]	0.67	0.01
	Glucose	1.03	1.43	1.03	1.56	0.06	0.34
1	Glycogen	79.29	65.24	76.99	70.45	2.37	0.13
	Lactate	27.43[a]	44.47[b]	29.87[a]	35.79[c]	1.74	<0.01
	Glucose	0.93[a]	2.58[b]	0.98[a]	1.62[c]	0.13	<0.01
3	Glycogen	58.98	51.35	61.18	56.61	3.26	0.65
	Lactate	52.29[a]	74.46[b]	53.34[a]	54.47[a]	2.53	<0.01
	Glucose	2.05[a]	4.03[b]	2.26[a]	2.59[c]	0.16	<0.01
10	Glycogen	45.48	37.91	47.56	42.7	1.76	0.46
	Lactate	68.8	82.9	68.84	72.27	3.18	0.11
	Glucose	3.33[a]	4.88[b]	3.68[a]	4.11[c]	0.21	0.02
24	Glycogen	38.21	38.27	44.77	39.67	1.39	0.08
	Lactate	84.01	88.91	82.05	83.62	0.94	0.10
	Glucose	4.74[a]	6.07[b]	4.34[c]	4.99[a]	0.09	<0.01

*Post stimulation treatment

[a,b,c] Means in the same row followed by different letters were significantly different

Appendix 7.5. Postmortem colour development in meat

Development of meat colour in the postmortem period prior to carcass grading depends on the concentration and chemical state of myoglobin, as well as physical properties of meat particularly as influenced by water content (Renerre 1990). Myoglobin (Mb) is the respiratory pigment that lends colour to meat. Its function is to reversibly bind to and facilitate diffusion of oxygen from outside the sarcolemma to mitochondria within the cell (Renerre 1990). The rate and extent of pH decline in postmortem tissue influence protein solubility, water binding capacity, and surface light reflectance (Renerre 1990; Murray 1995).

During the conversion of muscle to meat, muscle cells continue to respire, resulting in an intracellular accumulation of lactic acid and a concomitant decline in cellular pH. Both high temperature and high pH favour oxygen consumption by residual respiratory enzymes (Renerre 1990) within mitochondria that function efficiently at relatively high pH (Faustman 1994) approximating the physiological state. Because high muscle temperature will promote a rapid glycolytic rate and an increased rate of pH decline, the influence of temperature on enhanced mitochondrial function is valid only very early postmortem. The preferential consumption of oxygen by mitochondria results in limited binding of oxygen to Mb, producing, at the meat surface, only a thin surface layer of oxygenated Mb (Renerre 1990), the chemical state bearing a bright red colour (Faustman 1994). Additionally, a relatively high pH, greater than the isoelectric point of sarcoplasmic and myofibrillar proteins, will result in free water being tightly bound within the myofibres (Lawrie 1998). Bound water will act as a barrier to oxygen diffusion into the tissue, limiting the availability of oxygen for binding with Mb, and will result in tightly packed myofibrils (Lawrie 1998). Because the light scattering ability of an uncooked meat surface is partially dependent upon water binding capacity (Murray 1995), tissue with a greater water content will reflect less light than a sample with lower water binding capacity (Renerre 1990; Lawrie 1998).

When pH decline is rapid, mitochondrial function is inhibited and oxygen binds preferentially to Mb. Rapid pH decline also results in denaturation of the globin moiety of Mb, dissociation of oxygen from the haem group, and oxidation of Mb (Renerre 1990) to the brownish metMb state (Faustman 1994) causing a decrease in colour intensity. Low temperature, however, promotes penetration of oxygen into the tissue and an increased solubility in intracellular fluids, maintaining Mb in an oxygenated form (Renerre 1990).

It is apparent that colour development is a complex issue and is influenced by a multitude of environmental factors. The application of certain postmortem carcass treatments can exploit the conditions that affect colour development with the goal of producing a desirable end result. Applying carcass electrical stimulation during the dressing procedure, or elevated temperature conditioning during the cooling process accelerates glycolytic rate and results in an accelerated pH decline. Because mitochondria function less efficiently in a low pH environment, residual and solubilized oxygen in muscle tissue will bind to Mb. Rapid pH decline also results in lowered water binding capacity and greater water availability at cut surfaces. Ultimately, processes designed to accelerate postmortem tissue metabolism will result in lighter, brighter meat. Accelerated carcass chilling treatments, such as blast chilling, tend to stall pH decline by inhibiting the function of temperature sensitive glycolytic enzymes. Residual oxygen is consumed by respiratory enzymes leaving little oxygen available for binding with Mb. Rapid chilling tends to produce meat with a darker appearance.

Objective measurement of colour requires the definition of individual colours as point within a three dimensional colour space (Figure 7-1) (Murray 1995). The CIE (Commission Internationale de l'Eclairage) colour space contains three axes that, in combination, serve to define precise locations within the colour sphere. The L* axis defines lightness on a scale of 0 (black) to 100 (white); a greater value corresponding to increased lightness. The a* and b* axes describe shifts from green to red and blue to yellow, respectively. Values for a* and b* can be mathematically combined (equations 1 and 2) to produce Hue_{ab} and $Chroma_{ab}$, the preferred and more comprehensible colour descriptors representing common colour name and saturation, respectively (Murray 1995).

Equation 1: $Hue_{ab} = \tan^{-1}(b^*/a^*)$

Equation 2: $Chroma_{ab} = \sqrt{(a^{*2} + b^{*2})}$

With L* and $Chroma_{ab}$ held constant, an increase in Hue_{ab} value corresponds to a shift towards yellow-red while a decrease reflects a move towards purple-red. At constant L* and Hue_{ab} coordinates, an increase in $Chroma_{ab}$ describes a colour of greater intensity or saturation. Colour can be evaluated objectively using any one of several available types of portable surface reflectance meters that provide L*, a*, and b* output (Murray 1995).

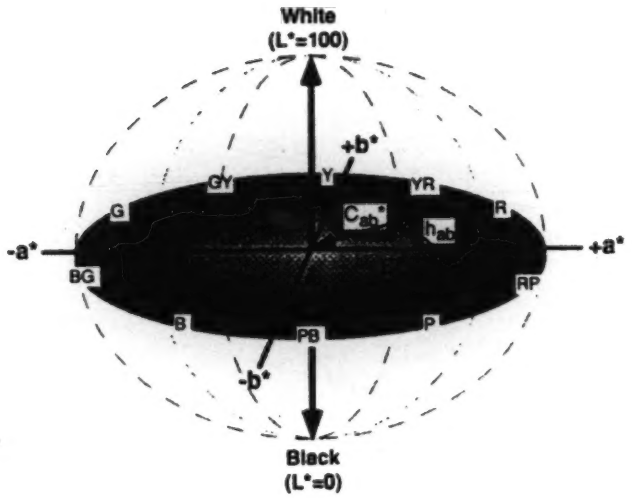

Figure 7-1: CIE L*a*b* colour space (Murray 1995)

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